

Calling the Tune and Paying the Piper

IN retrospect, the most striking feature of the six-month debate about the reorganization of civil research and development in Britain is the marvellous silence which has now followed the ending of the debate. The government has spoken, and although Mr Airey Neave and the House of Commons Select Committee on Science and Technology still reckon to have fish to fry, the research councils which are principally affected by last week's changes (see *Nature*, **238**, 124; 1972) are manfully, and even a little loyally, saying that they will try their best to make the new system work. To be sure, they would have preferred that the transfers of money from the budgets of the research councils to the customer departments of government had been smaller, but the £20 million now to be transferred is merely 70 per cent of the sum Lord Rothschild originally proposed, while the process of transfer will be spread over the coming three financial years. Given the government's assurance that no further changes of organization will be introduced within that period, this is something to be grateful for. But the white paper is, in any case, "sober, perceptive and intelligent" (*Nature*, **238**, 120; 1972) and with luck could become a better framework of organization than there has been for several years. That is something to look forward to.

The disagreement between the government and the Select Committee is chiefly a consequence of the way in which the committee's recommendations, themselves the product of the matutinal labours which count in parliament as midnight oil, were brushed aside in two casual paragraphs. Although there is much to be said for decisive government, in the aftermath of a debate that the government itself had begun, it would have been seemly if the Select Committee and other critics of the past few months had been given reasons why their arguments have been rejected. That said, the government was right not to follow the committee's view that there should be a minister for science and technology—the issue is simply whether science and technology must be considered parts of the ordinary machinery of government or a distinctive force, powerful but separate from the everyday departmental machinery. In the long run, it will be best for everybody if ministers can be persuaded that a department lacking competence in science and technology is a bad department. The government could, however, have been more generous to the Select Committee by acknowledging that its own determination to make the scientific policy of the departments more publicly accessible derives from one of the committee's recent reports. There is also much in what the Select Committee had to say about such questions as the need that the industrial research establishments of the Department of Trade and Industry should be given a measure of independence. This notion, if it had been implemented, could have avoided some of the artificial devices by means of which the government is now seeking to make the Department of Trade and

Industry at once a customer and a contractor (principally for itself).

The new proposals on the research councils leave several questions unanswered. According to last week's white paper, £10 million will be transferred from the research councils to the departments in April 1973, but although the departments have apparently begun to identify the particular research projects they will take over, there is hardly time for them to negotiate as between customers and contractors before the annual deadline for civil service estimates in October. At least two ministries, the Department of Health and Social Security and the Ministry of Agriculture, Fisheries and Food, will by then still be blessed with chief scientists still learning to find their way about their office filing systems, so that for the first of the three financial years, at least, the customers will be lame dogs to say the best of them. The true test of whether the government's version of Rothschild is functioning well will come only in the body of case law built up in the next eighteen months or so, usually around new projects.

During this period and beyond, there are two important and practical questions that will preoccupy the research councils, the first of which is capital expenditure. Last week's white paper says disarmingly that "expenditure on new capital facilities will be met by customer departments to the extent that the facilities are related to their requirements". Does this imply that if a customer's requirement entails the purchase of new capital equipment, or the building of a new laboratory, the customer will foot the whole bill but leave the research council in possession once the contract is completed? Or will government departments retain a lien on capital facilities which they have helped to pay for, with all the bureaucratic difficulties that divided ownership entails? What in any case will happen if, for one reason or another, equipment is delivered late or if buildings are finished late? As things are, research councils can usually adjust their programmes to make up for delays like these, but this will be more difficult when customers and contractors are locked in a contractual relationship. There is a case for asking that, in these special circumstances, the customer departments of government should pay the whole cost of the capital facilities which their requirements dictate, but that the contractors should thereafter retain possession. There will be obvious difficulties if government departments use their new freedom to spend money on commissioned research in ways that entail a substantial novel capital expenditure, at least within the confines of a limited budget. Will the research councils be expected to fire people so as to make room for the capital expenditure the customers require? That thought may not be unthinkable, but it is impractical.

Anxieties about the long-term security of employment with the research councils are more serious. According to the government's white paper, the research councils



will be at liberty to turn down research contracts for which they consider themselves to be inadequately equipped or which they consider it would be invidious to accept. In practice, however, a research council which decides to turn away a juicy contract will be jeopardizing the livelihoods of many of its employees. In circumstances like these, the councils can hardly be thought to be negotiating freely as contractors. Or does the government intend that the research councils, like other contractors, should be free to make provision for the loss of business from a powerful customer by spreading the risk, and by setting out immediately to win a reputation as research contractors in the commercial field? Plainly it would be helpful to all concerned if the government were now to say what plans it has for dealing with the painful situations that might arise if particular research councils find their work contracting or changing to such a degree in character that people are thrown out of work. No doubt this is a question that the Institution of Professional Civil Servants will be asking.

The declaration in the white paper that the Chief Scientific Adviser is now to begin an investigation of the way in which government departments can make their planning of research policy more explicit and more public is entirely welcome. But here again there are serious difficulties to be overcome. On the face of things, it would seem that the research contracts made between government departments and research councils acting as contractors, together with the detailed terms of reference, should be made public. But it is hard to see the departments following such a course for fear of the precedent that will be set for their dealing with other less disinterested contractors than the research councils. And although there is much that government departments can do to inform the public at large of what they are up to in research and development by issuing annual reports of work in progress, the conventional form of documents like these is no great help in professional assessment of the wisdom and the competence of the departments. In short, if the government is to implement the promise it has made, it will have to break ground that is new by British standards in publicity.

The most hopeful part of last week's white paper is the proposal that the Council for Scientific Policy should be reconstituted. The intention is that the present voluntary committee should be replaced by one consisting of the heads of the research councils, the chief scientists of interested government departments and independent members in roughly equal thirds. Although, on paper, the new council would be advisory to the Department of Education and Science, the white paper says that the council will also be required to monitor the way in which the new machinery for the administration of civil science is functioning. It follows that if the council is to do its job effectively, it will have to examine not merely the contractors (the research councils) but the customers (the government departments). In other words, there is at least a chance that the reconstituted Council for Scientific Policy will emerge as a powerful body able not merely to say amen to what ministers have already decided but to create new opportunities. Mr Airey Neave may not be as far away as he fears from the goal of a policy-making body in government science (which is not to say for a minute that there should be a minister as its chairman).

The most important gap in the government's proposals,

for which it is not directly to be blamed, is the lack of machinery for using government facilities and resources for meeting the needs of civil industry. Over the years, a ragbag of devices has grown up. The cooperative research associations, once the spearhead of government initiative in industrial research and development, are plainly headed for what they will consider the scrapheap. The National Research and Development Corporation, an invention of the 1940s, is destined to become the residual legatee of the wishes of previous governments to be kind to inventors. The laboratory at Harwell is busily making money on research contracts with industry, but nobody pretends that the success of Harwell can be replicated in places where there happens not to be an entrepreneur in charge. And the industrial research laboratories of the Department of Trade and Industry, shunted about from pillar to post as they have been, are unlikely to become an effective facility for meeting the needs of industrial research within the new contractors division of the Department of Trade and Industry. Then there is at present no way of guessing how the requirements boards which the department is setting up to formulate the research needs of important sectors of industry will function. These appeared like rabbits out of Dr Ieuan Maddock's hat when he gave evidence to the Select Committee earlier this year, but they have not yet been established. Much will depend on how widely they are briefed. If their role is merely to direct the work of the department's laboratories more effectively, to supervise the non-nuclear work of the Atomic Energy Authority and to oversee the department's contract work with industry and the universities, the device appears somewhat cumbersome. In the current financial year, the total cost of these activities is merely £26 million. But if the requirements boards are to be encouraged to look for new ways of spending money on industrial research and development within a larger budget, they could turn out to be more valuable. In that case, however, it is to say the least of it, surprising that the nationalized industries have been excluded from the present review of public expenditure on research. Why not a requirements board for telecommunications, for example? And if the Department of Trade and Industry does energetically set out to develop the new requirements boards, is it not likely to finish up by rediscovering the old industrial research and development authorities which have, in the past decade, popped up in one form or another in most proposals for the reorganization of publicly supported science?

This said, the scientific community should now make the best it can of the new arrangements. In the white paper last week, the government was at pains to emphasize its sense of partnership with the scientific community, and there is probably much in its belief that the Rothschild debate, niggling though it may have been in public, has served paradoxically to bring the research councils closer to the rest of the government machine than they have been since the 1950s. And there is no essential reason why governments should be at loggerheads with the organizations chosen as channels for public expenditure. Would it not be splendid if the research councils were now to become not merely ways of spending public money but ways by means of which the scientific community could influence government policy on research and development?

No PL 480 in Israel

ISRAELI universities have in the past few months been seriously downcast by the prospect that an important source of income for scientific research will dry up later in the year as a result of a largely administrative decision in Washington. Ever since the Food for Peace Program in the 1960s, authorized by what is known as Public Law 480, the United States Administration has been accumulating substantial amounts of foreign exchange in countries which were the recipients of foreign aid in the form of food. In making contracts under PL 480, the United States government would frequently find itself having to agree that payment should be made in local currency which, according to the local need for foreign exchange, could not afterwards be exported as hard currency. Both in developing countries such as India and in exceptional countries such as Israel, the US Administration has generously agreed that PL 480 funds, the property of the US government, should be spent locally on a variety of good causes—technical development of various kinds, the support of universities and other cultural institutions and the conduct of scientific research. But now the prospect is that the PL 480 programme will decline as the need for food as such in the pattern of foreign aid itself declines. Israel will be especially affected. The result will be that universities in Israel which have come to rely on funds like these as a source of income will be hard hit.

The Hebrew University in Jerusalem is a striking illustration of how the impending change will affect research. Although the running costs at the university come largely from the government of Israel and the Jewish Agency, most of the scientific research which the university carries out is financed by means of contracts and grants with outside bodies. Over the years, the research programme has grown at a healthy rate and the value of its contribution to international science has been conspicuous. In 1967, expenditure on research and development at the university was IL8.6 million (IL10=£1 sterling), of which the United States government supplied more than three quarters. By 1968, PL 480 funds amounted to IL9.8 million out of a total expenditure on research and development of IL14.1 million. 1969 saw an actual decrease of research funds because of a downward fluctuation in the amount of PL 480 funds available. In the current financial year, total expenditure on research and development is estimated to be IL23.2 million, consisting of IL6.7 million from PL 480 funds, IL7.2 million from the government of Israel and other local sources and IL9.2 million from foundations and other private sources, some in the United States. By all accounts, German foundations have been especially generous in their treatment of the Hebrew University, and German funds now account for 17.5 per cent of the research and development budget at the Hebrew University. Unhappily, however, the size of the US government's commitment to the Hebrew University in the current financial year is principally a consequence of the wish to spend the money available in Israel before the current financial year is over. The prospect for next year is bleak—probably there will be no PL 480 funds of any kind.

The first thing to be said, of course, is that Israeli

universities, like universities elsewhere, have been lucky to enjoy the benefit of PL 480 funds for as long as they have done. Over the years, the United States Administration has been exceedingly generous in its dealings with universities abroad, and the workings of the Food for Peace Program have provided a convenient umbrella beneath which activities like these could be sustained. But universities in Israel have to a substantial degree become dependent on this source of income for research and development and it is probably not an explicit part of the US Administration's intention that its declared policy on foreign aid to Israel as a whole should have the unfortunate consequence of imposing a sudden restriction on the scale on which Israeli universities can sustain programmes of research and development to which they are committed. So does it not follow, even at this late stage in the budgetary procedures of the United States, with the new fiscal year already begun, that the government agencies which have in the past had access to PL 480 funds should be enabled to work out with Israeli universities means of continuing the work which they have begun in the past few years?

100 Years Ago



Hereditary Instinct

WILL you allow me to recount to your readers what appears to me to be a striking instance of the transmission of impression in animals?

A few years ago I bought in Skye a perfectly uneducated Skye terrier. The first accomplishment which I taught him was that of "sitting up"—an accomplishment which he had great difficulty in acquiring. This was not owing to any stupidity on his part, for when he had once passed over this *pons asinorum* of dog-performances, he proved to be a very clever animal, and learnt many other tricks with great ease. He appears, however, never to have forgotten the pains which were taken to teach him his first trick, and to have judged therefrom that there is great merit in sitting up. Not only does he rely upon this as a last resource to move me to take him out, or not to whip him, but he judges that it must soften even the heart of an india-rubber ball. Sometimes when annoyed at his playing with this, his favourite toy, I have placed it on a chimney-piece, and turned my attention elsewhere. On looking round again I have seen my dog sitting up to the india-rubber ball, evidently hoping that it would jump down and play with him again. Perhaps he looks upon this ball as "animated by a living essence" (*vide* Chap. ii. of Darwin's "Descent of Man").

My dog is now the father of a family, and one of his daughters, who has never seen her father, is in the constant habit of sitting up, although she has never been taught to do so, and has not seen others sit up. She is especially given to this performance when any other dog is being scolded. Whether this is an instance of helping a fellow animal, of which Mr. Darwin gives such curious examples, or whether the dog simply hopes to avert the passing storm from her own head, the fact appears to me patent, that this dog has inherited the impression that sitting up has some special virtue for turning away wrath.

L. HURT

Alexandra Hotel, Harrogate, July 27

From *Nature*, 6, 261, August 1, 1872.

OLD WORLD

Select Committee Under Attack

THE House of Commons Select Committee on Science and Technology was in the wars last week. Within twenty-four hours it was threatening Mr John Davies, Secretary of State for Trade and Industry, seething with righteous indignation at Lord Jellicoe, the minister responsible for the white paper on research and development, and was itself attacked by the Confederation of British Industry. But it was the white paper which really angered the committee. Within minutes of its publication, Mr Airey Neave was expressing "extreme annoyance"—"it ignores fundamental questions", he said, "and the many conclusions and recommendations of the committee are contemptuously dismissed in two paragraphs".

Mr Neave is irked that the select committee has taken evidence for some months and rushed out its reports on government research and development because it believed that Lord Jellicoe had declared himself ready to wait for and consider its reports. As it is, the two paragraphs in the white paper devoted to the select committee's views do not give reasons why the government rejects the proposal for a minister for research and development, nor does it comment on how the government research establishments should transfer technology to industry or how the sums to be transferred from the research councils to government departments have been decided. Finally, the committee's recommendations on the non-reactor research and development carried out by the UK Atomic Energy Authority are not mentioned. As a result the committee has virtually summoned Lord Jellicoe to appear before it on August 2 when, Mr Neave said, Lord Jellicoe "will no doubt be asked to explain these and other matters".

The select committee will then report to the Prime Minister and ask for a statement on his attitude to the matter and his attitude to back-bench committees as a whole.

The select committee has also struck its own blow for open government by sending for the secret Docksey report on the future of the National Research and Development Corporation. Mr P. Docksey, former general manager of BP research and technology, started examining the exploitation of inventions resulting from public research and NRDC support in February 1971 and reported to the government in December. Demands that his report should

be published have been refused and the select committee, which is becoming increasingly worried about the number of government reports which are forming the basis of policy but which are not receiving public perusal (the Vinter report on thermal reactors is a *cause célèbre*), has decided to take action following a hearing last week at which Mr Docksey gave evidence.

Mr Docksey said that he believed that the NRDC should continue to function, but only in the university and industrial fields. Government establishments—the industrial research establishments and Harwell—should have a separate organization for exploiting inventions, although he emphasized that the two centres should cooperate closely. He also argued that the NRDC should not have to break even financially. It should fund innovations on an expenditure basis year by year, he said, and consider income separately, accepting the fact that financing inventions is unlikely to be profitable.

Questioned on publication of the report, Mr Docksey said that he did not consider his report a deep review of the subject and that if he had thought it was for publication he would have wanted to spend more time researching the subject. He said that the report is written informally and contains some confidential matter that might prevent publication. If this were edited out before publication, he said, "the report would be rather thin".

Nevertheless Mr Neave, declaring that the committee has power to send for "persons, papers and documents", said that the DTI would be asked for the report and if it failed to materialize the committee would "seriously consider what parliamentary action it can take". If the committee is successful it may well attempt similar tactics with the Vinter report.

Later the same afternoon, Mr Michael Heseltine, Minister for Aerospace, dashed any hopes that the committee's action might force publication of the report by declaring roundly that it would not be published. He added that Mr Docksey's chief recommendation had been that a development council should be established to review decisions to proceed with the development of an invention. This council was to be set up around the existing NRDC. The customer-contractor approach that has been evolved since Mr Docksey's report will, however, cater for this

continuous review, Mr Heseltine said, and therefore the council will not be set up. "The NRDC will continue to fulfil a role under the Development of Inventions Act, 1967, subject to further consideration of the scope of its relationship with the various government departments and other bodies concerned".

The committee's other skirmish of the day came with a formal statement from the Confederation of British Industry. It attacked the select committee's interpretation of its attitude to the industrial research establishments, which the committee recently described as "uncooperative and unconstructive", and criticized the conclusions published in the Select Committee's four reports. The CBI states that the appointment of a minister for research and development would weaken the links between the department's objectives and research and development programmes and would be unworkable. Further, the CBI claims that the committee underestimates the difference between pure and applied research, and states that "adoption . . . of the committee's mode of thought would . . . result in a setback to recent very welcome progress in the organization of government research and development".

RESEARCH COUNCILS

Wolf at the Door

HAVING been the most vociferous body in opposition to Lord Rothschild's recipe for reorganizing the research councils during the early months of this year, it is a little surprising that in its annual report, published this week, the Medical Research Council seems to have bowed to the inevitable.

Compiled before the appearance of the white paper on research and development, the comments on the reorganization of research are necessarily guarded. But the council says that whatever decisions the government takes there will be significant changes in the coming years.

The organization of the council's research boards in clinical research, tropical medicine and cancer research will be reviewed and the council says that it will aim at greater representation of government departments on its committees and working parties—a recommendation that is also contained in the

white paper.

The council only mildly rebukes the government for the way it allowed Lord Rothschild to carry out his inquiry into the research councils. It appeals to the government to institute a policy of periodic reviews but suggests that such reviews should only be carried out after the government has decided on its aims for research and on its policies for the universities. If there is a punch line hidden in the report it must be in the suggestion that reviews of the research councils "should include open discussion with the bodies involved".

On research, the council says that it is making special efforts to expand research on arterial disease, which includes coronary thrombosis, as well as drug misuse and dependence, mental health and population control. The council is also keeping a sharp eye on cancer research, infections and environmental factors in disease. The council also reports that it is encouraging the training of investigators in clinical neurology, clinical pharmacology, dentistry, dermatology, epidemiology, mycology, obstetrics and gynaecology and psychiatry.

The council received a grant from the government of £23.47 million in the year ending March 1972 but it also received an additional £1.62 million from private bodies and other sources. But this high water mark in the council's finances has been reached by annual increases (in terms of constant prices) which have decreased from a 13 per cent increase in 1967-68, when the total grant was £13.76 million, to only 3.7 per cent last year.

NUCLEAR POWER

No to Connah's Quay

THE CEBG plan to build a 2,500 MW nuclear power station at Connah's Quay on the river Dee has been rejected by the government. Following a public inquiry held at Mold last year, Mr John Davies, Secretary of State for Trade and Industry, in consultation with the Secretary of State for Wales, has decided to refuse consent, to the relief of Cheshire and Flintshire County Councils who had opposed the scheme.

The Dee is the centre of a redevelopment plan which involves the building of a barrage across the estuary followed by considerable urban growth in the area, and Shankland, Cox and Associates, who ran the studies for the proposed expansion, recommended that the application to build the power station be rejected as it would limit urban growth. The government's acceptance of this recommendation augurs well for the development scheme which could give the Flint side of the estuary a population of 280,000 by the year 2000

and provide water storage to yield 250 million gallons a day, as well as extensive recreational facilities.

The Engineering Inspector suggested at the inquiry that the application for the power station should be put in suspense until the full Deeside strategy is worked out, but Mr Peter Thomas, Secretary of State for Wales, argued

that this would impose unacceptable constraints on planning and development in the area.

The only fly in the ointment as far as those who want to see the Dee scheme go ahead are concerned is that although the application has been refused, another application may be made "if circumstances were to change".

Fischer Dominates

The first quarter of the Spassky-Fischer match in Iceland has now been completed with Fischer in the lead by $3\frac{1}{2}$ to $2\frac{1}{2}$ points. This state of affairs is quite different from what it was two weeks ago and Spassky has only managed to obtain one draw out of the last four games. Fischer has shown increasing confidence and incisiveness in his play while Spassky has exhibited a contrasting hesitancy and nervousness.

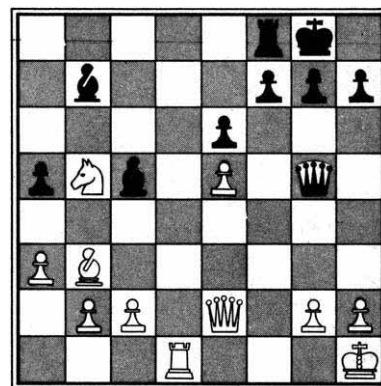
Spassky tried hard, however, in the fourth game and this has probably been the best contested game of the match so far. Fischer played his favourite variation against the Sicilian Defence, but it was soon evident that the Russian camp had prepared a strong counter-attacking line based on the force of black's two bishops bearing down onto white's king side at the cost of a pawn.

The diagram shows the position after white's 21st move of the fourth game. Spassky has the prospect of an attack and his next move aims to create some weaknesses around the white king. Fischer is driven on the defensive and offers the exchange of queens on his 25th move. Spassky boldly declines and thrusts his king's knight's pawn into action, on the 26th move making room to bring his rook into the attack on the king's rook file. Fischer has just enough time, however, to bring his knight back to the defence and compel black to simplify down to a drawn endgame.

The fifth game found Spassky apparently mishandling the white pieces once again (as in the third game, although this time the opening was a Nimzo-Indian Defence), and it was not long before Fischer was able to build up some pressure on the weak points of Spassky's position. It would be difficult to imagine that Fischer would necessarily win against a stubborn defence, however, and Spassky's blunder allowing a simple combination (by World Championship standards) came as a considerable shock to the Chess World.

The sixth game showed that Spassky had not fully recovered from his experience of the previous round and was outplayed in a game where Fischer seemed able to build up his attack at leisure. This game was all the more demoralizing for Spassky in that he was permitted to play a defence with which he has had a good deal of experience whereas Fischer had never been known to encounter it before. Spassky will probably need some good fortune in the next game or so in order to regain composure. J.P.

SPASSKY BLACK



WHITE FISCHER

Position after white's 21st move of the fourth match game

THE FOURTH GAME White: Fischer Black: Spassky Sicilian Defence

White	Black	White	Black
1 P-K4	P-QB4	23 B-B4	P-KR5
2 N-KB3	P-Q3	24 P-R3	B-K6
3 P-Q4	PxP	25 Q-N4	QxP
4 Nxp	N-KB3	26 QxRP	P-N4
5 N-QB3	N-B3	27 Q-N4	B-B4
6 B-QB4	P-K3	28 N-N5	K-N2
7 B-N3	B-K2	29 N-Q3	R-R1
8 B-K3	0-0	30 N-B4	BxN
9 0-0	P-QR3	31 QxB	B-Q3
10 P-B4	NxN	32 Q-B3	QxQ
11 BxN	P-QN4	33 PxQ	B-K4
12 P-QR3	B-N2	34 R-Q7	K-B3
13 Q-Q3	P-QR4	35 K-N1	Bxp
14 P-K5	PxP	36 B-K2	B-K4
15 PxP	N-Q2	37 K-B1	R-QB1
16 NxP	N-B4	38 B-R5	R-B2
17 BxN	BxB ch	39 RxR	BxR
18 K-R1	Q-N4	40 P-QR4	K-K2
19 Q-K2	QR-Q1	41 K-K2	P-B4
20 QR-Q1	RxR	42 K-Q3	B-K4
21 RxR	P-R4	43 P-B4	K-Q3
22 N-Q6	B-R1	44 B-B7	B-N6
		45 P-B5 ch	B-N6

Drawn by agreement

Was this Debate in Vain?

This comment on the recent white paper on research and development is by John Lyons, deputy general secretary of the Institution of Professional Civil Servants.

ONE is bound to wonder why the government went to the trouble it did—and it went to a lot of trouble—to consult all the interested parties about the Rothschild Report when, to judge by the result, it had so little intention of departing, other than marginally, from Lord Rothschild's main tenets and recommendations. The trouble was that the government had accepted Lord Rothschild's chief recommendations before the report was published. It is not surprising that there is no reference to the first recommendation of the Select Committee that "the consultative nature of . . . a green paper should not be impaired . . . by government statements of policy in advance".

The original impression Lord Rothschild's report made was that the working scientist should be happy to work at the bottom of a command structure headed by "the customer". This was a concept which the Institution of Professional Civil Servants challenged particularly strongly. It is pleasing therefore to see the government state that provision for continuing discussion and partnership between customers and contractors is essential; and that "in practice many of the ideas for research and development to meet the customer's needs come from the scientific staff in the contractor's organization". True, this still understates the real position but it is nevertheless welcome.

The government shows a welcome flexibility in the organizational arrangements it has allowed departments to develop. Some commentators have criticized this, but it would have been ridiculous to have tried to force every department's research and development into an iron organizational mould. This does not mean, however, that anything goes. Whatever the precise form in any department, the chief scientist's organization must be capable and effective, have the means to do the job, and be strong in the department's counsels on all matters in which the voice of science should be heard. It is by no means certain yet that that situation will obtain in MAFF, which is to acquire £10 million of ARC funds by 1976. Discussions are continuing and all may yet be well. In this connexion the statement in paragraph 51 of the white paper that no transfers of money from research councils will be made "until

the customer departments have established their central scientific staffs" is not good enough. On the face of it, it contrasts badly with the statement of the Lord Privy Seal in the House of Lords on February 29 that the government had "a closed mind . . . on the principle that no transfer should take place unless and until the government departments concerned are properly equipped and capable of managing the funds entrusted to them". Obviously, the statement in the white paper must be interpreted in the light of the Lord Privy Seal's statement.

As widely anticipated, the government rejects the main recommendation of the Select Committee on Science and Technology that there should be a Minister for Research and Development with his own vote. Instead, the government restates its belief that applied research and development is a function of other policies, not an end in itself. There is much strength in this view and it has needed stating. But one does not have to go all the way with the select committee to think that the government is going too far out on the limb of functionalism. There really is more for the Chief Scientific Adviser to do than to look after inter-departmental coordination and advise ministers on the scientific and technological aspects of the government's policies. Somebody, for example, needs to be concerned with the total balance of the government's research and development effort. Was it, for example, really a matter for the responsible department at the time to decide by itself that so vast a proportion of the government's research and development funds for civil purposes should go into Concorde? Is research into environmental problems to be determined solely as a by-product of the political objectives—however good—of the Minister for the Environment of the day? How will the government deal with Europe, which is clearly interested in having a research and development policy as such? These doubts do not of themselves support the particular proposal of the select committee, but they do suggest that the government's reaction to that proposal is inadequate.

The section on the role of scientists in government management and administration is a valuable re-affirma-

tion of the government's policy of bringing more people with a scientific or other professional or specialist background into general management and administration. Nevertheless, it lacks bite. On measures to bring about the desired end, the white paper only records what is already happening. There is no reference to the overdue need for departments to review the procedures for appointments to posts at Under Secretary and above so that the quite large numbers of existing scientists and professional staff at senior levels are properly considered for promotion to them. The situation in the Department of Trade and Industry, for example, cries out for reform. The proposal to set up a "task force" to study the problems of mobility of scientists as between the civil service, research councils, universities and industry and so on is a good one, and long overdue. The "task force" would be strengthened, however, if it included someone from the staff association side. We have as much experience of the general problems of transferability as anyone.

When one comes to the research councils on the central issue of financing, the government has gone 75 per cent of the way with Lord Rothschild. Why? We are not told. The figures setting out the income that is to be transferred are as arbitrary as Lord Rothschild's own.

Nor would anyone know from reading the white paper that there existed a concept known as "strategic science" around which much of the debate about the research councils raged. It is true that an understanding of the concept, first advanced by Sir Frederick Dainton, is essential to a proper understanding of the role of the research councils, but this is another minor matter not worthy of a mention. What we are witnessing is a straightforward transfer of funds from the research councils to departments because that is what the government decided to do in the first place. There is simply no point in looking for sophisticated explanations. The manner of transferring the income from the research councils to departments—over a three-year period—is nevertheless a distinct administrative improvement on the original proposal. It gives some time for both parties to adjust to the new situation. The insistence throughout the white paper that the new relationships must all be based on partnership and cooperation is also important. Without this the new organization will not work. It is because of this emphasis on partner-

ship that the statement that "no conditions will be placed on the money transferred to customer departments, but the expectation is that it will be spent to commission applied research work from the research councils" is perhaps not as weak as it sounds. Nevertheless, staff will need to have assurances that there are no intentions by departments suddenly to cut back on the amount of their commissioned research with the research councils; departments must plan their commissioned research with the research councils on the basis of five-year rolling programmes.

The most important paragraphs about the research councils are those concerning the new Council for Scientific Policy. The government has got the composition right. And it has strengthened its authority. That the CSP "will know and be able to take into account the size and nature of the

work to be commissioned by the customer departments", and that the government will consider its recommendations in planning future totals of public expenditure on research and development, is not only a safeguard to the research councils and the universities, but potentially a means of bringing a wider viewpoint to bear on government decisions about civil research and development which could be of great benefit to all parties concerned.

There is a host of detailed matters to be cleared up in the wake of the white paper. Some of these will be of major significance in determining how things work out. The government has placed the greatest possible emphasis on partnership and cooperation with and between the various parties. Everything will depend on that spirit being an integral part of the package throughout.

vancy, including the management of nature reserves". All other recommendations for reorganization have also advocated keeping research and management together. Thus in 1969 the Select Committee on Science and Technology, considering the position of the conservancy in NERC, stated firmly: "All the evidence we have heard obliges us to conclude that conservation and research must go hand in hand, and that on balance it is desirable that they should be kept together under one body, even at the risk of compromising the Haldane principle". Again, in 1972, the Lucas Committee, enquiring into relationships between NERC and the conservancy, said that although at the outset some of its members had doubts about the wisdom of combining the two branches of the conservancy, all were won over by the evidence to support the existing situation, and they recommended as follows: "Effective conservation, in the present state of knowledge of the management of plant and animal communities, demands increased knowledge, so that conservation and research functions should be closely associated; these two Nature Conservancy functions should not be in different organizations".

Recently the conservancy has been involved in "A Nature Conservation Review" in which sites of ecological importance throughout Britain have been listed and evaluated. This is an essential blueprint for Britain's future conservation programme and for the safeguarding of potential nature reserves. The review was produced by the cooperative efforts of conservation and research branches; had they already been divorced, it could not have been written. How fortunate that it was completed in time!

It would be wrong to gloss over the differences that have occurred between the conservancy and NERC. Most of the staff, and most conservationists, regretted the loss of its independent research council status when it was absorbed in NERC, and attempts to regain this independence have been made. Recently, however, when this solution appeared to be impossible, closer integration within NERC of an undivided conservancy has been increasingly welcomed. It has been realized that the semi-independence of the conservancy has weakened NERC, and conservancy staff were, at last, coming to agree with the Lucas Committee that a strong NERC could foster all sides of conservation if all worked together to that end. The proposals in the white paper come at just the wrong time. They will do much to destroy the enthusiasm of those who have made the reputation of the Nature Conservancy so respected throughout the world.

Requiem for the Nature Conservancy

This comment on last week's white paper is by a government scientist.

ALTHOUGH all research supported by the government will, eventually, be affected if the recommendations of the white paper, *Framework for Government Research and Development* (Cmnd. 5046) are implemented, the only body which will be totally changed is the Nature Conservancy. The conservancy has, at present, two sides of almost equal size. The conservation branch is described in the white paper as being responsible for "the management of national nature reserves, and advisory, educational and protective work on wild life conservation". The other side, the research branch, is said to engage in "research, part . . . directly applied to the solution of conservation problems". The proposal now is to abolish the Nature Conservancy in its present form, leaving only the research branch within the Natural Environment Research Council, mostly financed by that council, but with some 30 per cent of the research funds transferred to the Department of the Environment, which will place research contracts, possibly with NERC. The conservation branch is to be transferred bodily to the Department of the Environment, under a new and undefined Nature Conservancy Council established by the Secretary of State and subordinate to his department.

The white paper's descriptions of the branches of the conservancy are not strictly accurate. As was pointed out

by Dr Kenneth Mellanby in his introduction to the recent Monks Wood Experimental Station report (*Nature*, 237, 246; 1972) the research staff is in fact deeply involved in both advisory and educational work, and any distinct divisions between research and conservation are meaningless. Almost all members of the research staff of the conservancy value this association with conservationists, both in their own organization and, increasingly, in voluntary bodies. This association results in a unique enthusiasm for conservation, and for the application of the results of conservation research, which would not otherwise exist. There is also a feedback from these contacts which encourages the research scientists to direct their efforts so as really to help with practical conservation.

The writers of the white paper were not the first to be worried because the Nature Conservancy does not fit neatly into the accepted pattern of a research council. The Slater Report in 1962 advocated a split along similar lines. When, however, the Trend Committee gave fuller considerations to its proposals in 1963, and when it implemented the suggestion that the Natural Resources Research Council (later renamed Natural Environment Research Council) should be set up, the first responsibility allotted to this new council was to include "all the functions now exercised by the Nature Conser-

NEW WORLD

Exploration from the Sky

ON Sunday this week, NASA launched the first of its Earth Resources Technology Satellites (ERTS-1) into a polar orbit lying between 561 and 570 miles above the Earth. There seems no doubt that the satellite is the most sophisticated of all data handling and data processing laboratories to have been put into an Earth orbit so far. But it is widely regarded by sceptics and cynics alike not so much as the first opportunity to harvest information about the surface of the Earth by remote control but, rather, as the first opportunity to test NASA's claims in recent years that Earth resources satellites are potentially even more profitable than telecommunications satellites.

ERTS-1 will function in two ways. First, it will collect data about local conditions relayed from 150 points on the surface of the United States by as many ground-based automatic sending stations, equipped to record such measurements as rainfall, snowfall, temperature, soil moisture and wind velocity. Second, the satellite is equipped with three television cameras sensitive to distinct spectral frequencies as well as with a four-channel spectral scanner recording light intensity, with a resolution of 80 yards, in the green, red, near and far infra-red regions of the spectrum. The total weight of the satellite (on the surface of the Earth) is 1,800 pounds and the technology of the device owes much to the development of the Nimbus meteorological satellite in the past few years.

The problems of data handling are of several kinds. If the resolution of the four-channel sensor were to be 100 feet—and the performance of the instruments in ERTS-1 is less good than this—the recovery of all this information would require the transmission of more than 200 megavits per second of information to the Earth. As well as the multispectral sensor, however, ERTS-1 is equipped with three television cameras which will between them produce some hundreds of thousands of television pictures each day. Storage within the spacecraft, which will be accomplished by magnetic videotape, is itself a problem, but S-band communication channels are also of limited capacity, and ERTS-1 will transmit merely 60 megavits per second.

NASA has made arrangements with groups of investigators in more than 30 countries to have access to the information collected by ERTS-1. Although it will be possible to identify in principle

the fields of individual farmers in the images collected, identification will to some extent be complicated by the departure from circularity of the orbit achieved last week. And although, at a conference organized by the Panel on Science and Technology of the House Committee on Science and Astronautics earlier this year, several visionaries looked forward to the time when the visual skills of the photogrammetry experts would be replaced by automatic comparisons of photographs, for so long as NASA and its helpers are trying more specifically to define what Earth resources satellites should look for, there will be no escape from visual comparison.

The immediate benefits of the Earth resources satellites are not easily foreseen. Plainly they will be objective tests of the long-held dream of the meteorologists that it will be easier to collect information about the weather by means of satellites. There should also be a useful yield of information about macroscopic questions—how much of the Earth is really covered by forest, or by snow? The television cameras will provide plenty of grist for the mills of those who would apply in civil fields what the military intelligence people have been doing, all these years, with the photographs returned from the surveillance satellites. Synoptic geology of out-of-the-way places should be stimulated (but in the last resort there may be no substitute for the man with a hammer). And the oceanographers have been promised a chance of learning much more than they know at present about such things as the distribution of plankton near the surface.

SICKLE CELL ANAEMIA

Programme in Progress

PRESUMABLY as a foretaste of things to come, the Department of Health, Education and Welfare has announced the award of \$9 million to boost research and community service on sickle cell anaemia. This money comes out of the funds made available for fiscal year 1972, after President Nixon's call in his health message to Congress in February 1971 for a \$5 million increase over the \$1 million already being spent, and the subsequent addition of a further \$4 million. The improvement should continue, for the National Sickle Cell Anaemia Control Act, signed into law by Mr Nixon last May, authorizes the expenditure of \$25 million in fiscal year 1973, \$40 million in 1974 and \$50

million in 1975 (see *Nature*, 234, 377; 237, 68; 1972).

The activities that are to benefit from the awards come under the auspices of the National Sickle Cell Disease Program, set up in response to the President's health message, and coordinated by Dr Rudolph Jackson, a haematologist who also heads the Sickle Cell Disease Branch of the National Heart and Lung Institute. All aspects of the battle against the disease are intended to benefit, and the \$9 million is to be distributed between nineteen screening and education clinics, ten comprehensive research and community service centres and thirty-four pure and applied research projects. The clinics will provide screening and detection facilities, as well as counselling and education programmes for 10,000 to 20,000 people each per year.

Haemoglobin S, which is responsible for sickle cell anaemia and was first described in 1949, can be detected by an electrophoretic test. Thus diagnosis is easy, and distinguishes between sickle cell anaemia (the homozygous condition) and sickle cell trait (the heterozygous condition, when there are no severe symptoms but the offspring are at risk of inheriting the disease). An important aspect of the work of the clinics will therefore be to counsel prospective parents.

In the United States, sickle cell anaemia affects chiefly the black population, and there are some fears that genetic counselling represents a form of genocide, although such fears are obviously groundless because counsellors are trained not to tell people whether they should or should not reproduce but to advise them of the possible consequences. To allay these and other fears will be a vital part of the educational activities of the clinics, which are to share about \$2 million of the awards.

The research and community centres, which share about \$4.5 million, are intended to combine fundamental research and clinical application with community services. Because sickle cell anaemia is genetically determined there is no cure, and clinical research is concentrated on improving methods of treating the painful crises that afflict the sufferers. Dr Jackson was confident last week that the programme which he is coordinating will decrease the frequency, morbidity and mortality of sickle cell anaemia in the United States—this, he says, is not genocide but rather "genosave".

NEWS AND VIEWS

Towards a Unified Theory of Muscle Contraction

CONSIDERABLE advance has been made recently towards an understanding at the molecular level of how the contractile activity in muscle is regulated. The basic mechanism of muscle contraction cannot yet be fully articulated, but during the past year or so a fundamental new element has been detectable to researchers in the field (see *Nature New Biology*, **238**, 1; 1972); namely that the experiments of structuralists, biochemists and mechanists have become directly relevant to each other. With a little thought it is possible for the different specialists to attempt to measure the same thing, be it numbers of myosin heads interacting with thin filaments, distance moved by myosin heads or muscle efficiency. It may be possible in the next few years to look forward to a unified explanation of muscle contraction; but this will have to include the regulation of muscle contraction; it is clear that considerable regulation is required to make muscles useful to animals. With reports such as that by Bremel and Weber in this week's issue of *Nature New Biology* (**238**, 97; 1972) the regulation story is to some extent running ahead of the story of the origin of force development in muscle and it looks as if studies on regulation can now throw light on the mechanism of contraction.

Muscle contraction takes place when a thick and a thin set of filaments are actively moved past each other. The origin of the shear force is not yet certain but the most likely source is the myosin heads which project in helical arrays from the surface of the thick filaments. In the presence of ATP and absence of calcium the muscle is relaxed and readily extensible; in this state the myosin heads are close to the thick filaments and do not attach to the thin filaments. In the absence of ATP all or most of the myosin heads move out and attach to the actin subunits of the thin filaments thus binding the two sets of filaments together and rendering the muscle inextensible—in rigor. In biochemical terms myosin is enzymatically active as an ATPase. The ATPase activity of myosin is increased many times in the presence of actin, and Ebashi and his colleagues found that the low ATPase in relaxed muscle was attributable to a relaxing system comprised of the proteins tropomyosin and troponin on the thin filaments. This inhibitory effect of the tropomyosin-troponin is abolished in the presence of calcium (above about 10^{-5} M), the ATPase of myosin is enhanced by actin and muscle contraction takes place most likely by cyclical attachment and detachment of myosin heads to and from actin.

How is this regulation brought about? The incisive experiments by Bremel and Weber provide answers to at least some relevant questions. They have considered that actin monomers in the thin filament may be either "turned off", in which case they are unable to interact with myosin heads in the presence of ATP, or "turned on". Troponin has at least two classes of binding sites for calcium, one of high and one of low affinity. When calcium is bound by the low affinity sites the effect is that actin is "turned on". One troponin molecule in conjunction with a molecule of tropomyosin is apparently capable of "turning on" seven actin monomers.

In the first set of experiments the binding of calcium to troponin was measured at two different concentrations ($8\text{ }\mu\text{M}$ and 2 mM) of MgATP. At the lower MgATP concentration there is an increased affinity for calcium and this suggests that the increased affinity could be the result of a change in the properties of the troponin molecule mediated by the formation of rigor complexes between myosin heads and actin at the lower MgATP concentration. This suggestion is supported by the second set of experiments. Synthetic regulated thin filaments were prepared—that is, thin filaments containing actin, troponin and tropomyosin—and the binding of calcium to troponin in these filaments was measured in the absence and in the presence of myosin. The calcium affinity of troponin was greater in the presence of myosin as a result of the increase in affinity of the low affinity sites just as in the first set of experiments when the ATP concentration was lowered to $8\text{ }\mu\text{M}$. Interaction of myosin heads and actin monomers can, therefore, alter the properties of the troponin molecule, but it is not possible to say whether the change is mediated through a specific few, all or only one of the actin monomers.

In the third set of experiments the actin activated ATP hydrolysis of an active fragment of myosin (S-1) was plotted against ATP concentration both in the presence and in the absence of calcium. Furthermore, two different actin and myosin fragment concentrations were used. From these experiments it is possible to argue that the formation of rigor complexes elicits a cooperative response from the actin filaments which "turns on" all the actin monomers in the absence of calcium. A threshold rigor occupancy is required to do this and this is tentatively estimated at four or five rigor complexes per unit of seven actin monomers.

Various mechanisms can be put forward to explain these results and a particularly promising one comes from the recent information about the structural changes which the thin filaments undergo during active muscle contraction. X-ray diffraction and electron microscopic studies by several workers now indicate that the troponin and tropomyosin molecules lie along the grooves of the two-stranded actin helix of the thin filaments. In resting muscle the troponin-tropomyosin strands appear to lie close to the actin helix, but in rigor when the myosin heads are attached to actin, the troponin-tropomyosin is displaced towards the centre of the groove (see *Nature New Biology*, **238**, 1; 1972). During muscle contraction the troponin-tropomyosin is also towards the centre of the groove.

The effect of the troponin-tropomyosin may therefore be to block the acto-myosin interaction in the absence of calcium. Calcium would then be seen as bringing about a conformational change in the structure of the troponin which causes the troponin-tropomyosin to move in the helical groove and unblock the access of myosin heads to actin. The X-ray studies which reveal this change may also show that there is more troponin-tropomyosin moved than would be required to unblock a length of thin filament proportional to the number of myosin

heads attached at any one time because this last fraction is less than 40 per cent. The "cooperativity" may be caused by nothing more than a tropomyosin molecule moving *en bloc* and simultaneously exposing several actin monomers.

Kinetic experiments on the sequence of reactions associated with ATP hydrolysis indicate that ATP is hydrolysed when the myosin head is not attached to actin. Thus the

"relaxed" state, often thought of as a passive state of muscle, is revealed as a rather special situation of probably high energy potential. The last word on regulation has still to be said, for there are indications from X-ray studies that the thick filament structures are also changed by calcium binding, but the present convergence of results from diverse techniques encourages the thought that ideas are on the right lines.—A. M.

Chemical Control of Behaviour

It may seem surprising that so much of this issue of *Nature* is devoted to the isolation, identification and synthesis of a compound which apparently causes rats to avoid the dark (pages 198 to 210). Contributors and readers are assured that this will not be a regular procedure. But the chemical control of learned behaviour, and its reported transfer from one animal to another, is a subject so fraught with pitfalls that it seemed necessary, while publishing the results of Dr Ungar and his colleagues, to set out clearly the reservations that many people will doubtless wish to voice among themselves. For this reason the report by Drs Ungar, Desiderio and Parr on page 198 is followed by an assessment of their data and conclusions by Mr Walter Stewart, a chemist who has no personal interest in the controversy except that he was asked to referee the report.

Fifteen months ago Dr Ungar and his colleagues submitted a short article which was a sequel to the report that the brains of rats trained to avoid the dark contain a substance which, when injected into naive mice, causes them to avoid the dark (G. Ungar, L. Galvan and R. H. Clark, *Nature*, **217**, 1259; 1968). In the second article, Dr Ungar and his colleagues announced that they had isolated the compound responsible for avoidance of the dark, determined its structure, prepared it synthetically and demonstrated that the natural and synthetic compounds have equal activity.

After reading the report Mr Stewart felt that, although clearly intended as a preliminary communication, it should be extended to include certain of the data on which

the authors had based their conclusions. Only then, it seemed, would a fair assessment of this work be possible. In two subsequent revisions, the authors enlarged their article to include some of the data which had been requested, but it became clear that Dr Ungar and Mr Stewart disagreed both on the amount of data needed to support the conclusions and the interpretation of the data already provided.

At this stage it did not seem desirable to delay publication further while seeking closer agreement between authors and referee. This work has already received a great deal of attention in the American press, and clearly it will be of great importance if eventually substantiated. And so the findings of Ungar *et al.* are published today, followed by Mr Stewart's detailed comments, embodying those of his reservations which remain unresolved. Dr Ungar and his colleagues agreed to reply to Mr Stewart within five days of seeing his comments, and their response appears on page 209. It is hoped that this unusual procedure will air the varied facets of a controversial topic and help readers to draw their own conclusions.

TUMOUR VIRUSES

SV40 Proteins

from our Cell Biology Correspondent
MODELS which account for the lysogenization of *Escherichia coli* by lambda and other temperate phages have always dominated the ideas which tumour virologists have about the mechanism of transformation of animal cells by the small DNA tumour viruses, simian virus 40 and polyoma virus. And, of course, the two processes, lysogeny and trans-

formation, have so many striking parallels that it is perhaps as inevitable as it may be misleading immediately to interpret any new observation of the biology of these two viruses in terms of the biology of lambda phage. Kaplan, Wilbert and Black (*J. Virol.*, **9**, 800; 1972), for example, have discovered that purified SV40 particles have an associated endonuclease activity capable of converting form I double stranded, covalently circular and supercoiled SV40 DNA into a relaxed form by cleaving a phosphodiester bond in either one or both strands of the form I DNA, and they draw attention to the fact that the lysogenization of *E. coli* by phage lambda depends on an endonuclease specified by the lambda genome.

Whether or not the endonuclease associated with SV40 particles is specified by the SV40 genome and has any role in transformation are entirely open questions. The presence of a nuclease, however, is hardly questionable; it nicks form I DNA without liberating any acid soluble nucleotides and probably at a few specific sites. The activity is dependent on magnesium ions, is heat labile and has a pH optimum between pH 6.7 and pH 7.1. Last year Cuzin, Blangy and Rouget reported in *Compte Rendu* a similar activity associated with preparations of polyoma virus, but in spite of the assurances of both groups that the virus preparations they used were free of contaminating host cell proteins they may find it hard to convince other workers that this activity is not some contamination. And even if they achieve that they still face the problems of determining the location of the activity in the virions and its function during infections.

The genomes of SV40 and polyoma virus are, of course, very small; they can code for only about 200,000 daltons of polypeptide, and so it would seem that determining the various proteins specified by so small a genome is a comparatively simple exercise. Of course, in fact the opposite is the case because the host cells are not only extremely complex but also continue to make RNA and protein while supporting the replication of these viruses. As Anderson and Gesteland (*ibid.*, 758), however, have now shown it is possible to detect in extracts of monkey cells supporting the growth of SV40 the appearance of three SV40 capsid proteins, VP1, VP2 and VP3. They have simply electrophoresed in SDS polyacrylamide gels extracts of whole infected cells and find that the three capsid proteins are made in detectable amounts only after the onset of replication of the viral DNA, at which time, of course, new virus-specific RNAs not present early in the infection can first be detected.

Anderson and Gesteland have also

detected a fourth protein (IVP 3.5) which appears in infected CV1 and BSC1 cells after the start of viral DNA replication but cannot be detected in extracts of infected MA-134 cells. They estimate that this protein has a molecular weight of about 15,000 and speculate that it may well be a degradation product of the major VP1 capsid protein.

Presumably the three capsid proteins VP1, VP2, and VP3, appearing after the onset of DNA synthesis, are specified by the viral genome. If this is the case and if the three proteins are unrelated they account for almost half the SV40 genome (98,000 of 200,000 daltons of polypeptide) which should considerably simplify the search for the early viral proteins made before viral DNA replication and including presumably the transforming gene product(s) and perhaps the SV40 T and U antigens. Anderson and Gesteland are obviously sanguine about their chances of detecting these early proteins by the techniques they have at hand. Success would indeed be a major achievement.

MUSCLE

Constructive Roles

from our Molecular Biology Correspondent
ONE of the most felicitous characteristics of the muscle field in recent years is the manner in which the structural approach has struck sparks from the biochemistry. Concerning the myofibrillar elements, other than actin and myosin, and to a lesser extent the proteins of the relaxing system, however, a state of considerable ignorance still persists. Several relatively major protein components have been identified, whose function is entirely conjectural, and whose presence in the filaments has served only to obfuscate structural analysis. Two studies in the current literature offer the beginnings of a chemical and biochemical characterization of two of the obscurer myofibrillar proteins.

Marimoto and Harrington (*J. Biol. Chem.*, **247**, 3052; 1972) have purified a protein, which originates in the dense zone, the M-line, in the middle of the A-bands, that contain the myosin filaments. In the M-line it seems that the thick filaments, packed in their hexagonal array, are tied together by bridges, in a system which must accommodate the expansion of the thick filament lattice that accompanies contraction. It has been surmised therefore that the M-line structure serves to maintain the register of the thick filaments in the lattice.

Marimoto and Harrington have identified their protein in immunological terms: an antiserum against a crude M-line preparation, resulting from extraction with low-ionic strength buffers, stabilizes the M-line in glycerol-

extracted fibres against such low-salt extraction procedures. If, on the other hand, the relevant antibodies were first absorbed out of the serum with purified M-line protein, the M-line in the fibres could no longer be fixed by the residuum. The purified protein proved to be a dimer, containing two apparently identical chains of some 43,000 molecular weight each, as measured by sedimentation equilibrium. In hydrodynamic terms the native protein is not highly disymmetric, nor does it have a high content of α -helix.

The molecules show no self-associating tendency, which argues against their association in the M-line bridges. They do, however, interact with myosin filaments: in the ultracentrifuge, in conditions that lead to an equilibrium between myosin with its filamentous aggregate, the M-line protein can be seen to generate a new rapidly sedimenting complex by combining with these filaments. In the electron microscope too, large and rather ill-defined aggregates are observed. It seems then that the properties of the M-line protein are indeed consistent with a structural role in the myofibrillar scaffolding.

Another hitherto rather poorly characterized muscle protein, which was first discovered eight years ago by Ebashi, is α -actinin, and this again has now been prepared in a state of high purity. Goll *et al.* (*J. Mol. Biol.*, **67**, 469; 1972) have achieved a homogeneous preparation, and have re-examined the interaction with F-actin, which is its only distinctive characteristic. One of the principal findings is that the nature of this interaction is markedly modified according to whether the experiments are done at 0° C or nearer the physiological temperature. Whereas at 0° C low molar concentrations of actin cause a steep rise in the viscosity of F-actin, the effect at 37° C is much less dramatic, and requires high actinin concentration. At 0° C, moreover, the interaction is little, if at all, affected by the presence of tropomyosin, a protein known to associate with the actin filaments, and sedimentation experiments show that the actinin actually displaces tropomyosin from F-actin.

Measurements of binding stoichiometry are fraught with problems, but the data may be said to support the contention that one actinin molecule, with a molecular weight of perhaps 200,000, binds firmly for every ten or eleven actin monomers, which happens to be the number in a turn of the thin-filament helix. The suggestion then is that the actinin is a bifunctional species, which links two actin filaments wherever they fall in a sterically appropriate register, which will happen once per turn. Such cross-linking will cause the porridge to congeal, in the manner observed.

By contrast, at 37° C the binding of actinin to F-actin is much less pronounced, and in addition it is chased by tropomyosin to the extent that only of the order of one actinin molecule for every 200 actin monomers remains undisplaced. If, as is believed, the actinin is localized in the Z-disks, in which the thin filaments are rooted, this makes good sense, for it would correspond well enough to the terminal association of one actinin molecule with each strand of the double F-actin helix. In a second article, Stromer and Goll (*ibid.*, 489) give tangible evidence for this scheme in the form of electron microscope pictures. Fibres extracted with low-salt buffers to eliminate Z-line material, and incubated with α -actinin at 0° C, showed a tracery of fine bridges all along the thin filaments. When the incubation was done at 37° C the new connexions appear almost entirely in the Z-disk region, at the ends of the thin filaments. Tropomyosin also generates little tufts in this zone, and diminishes the density of the actinin bridges. At 0° C the tropomyosin has no visible effect on the actinin mesh along the thin filaments.

A moral that is perhaps worth drawing from all this is that the myofibrillar proteins are sticky objects, designed to slot into lattices with many interaction sites, and that it may often be imprudent to impute any physiological significance to structures of complexes, except under closely defined conditions.

METEOROLOGY

Modifying the Weather

from a Correspondent

THE third conference on weather modification of the American Meteorological Society opened on June 26 in Rapid City, South Dakota, seventeen days after a disastrous flash flood that caused more than 230 fatalities. It was of considerable concern to the participants that there had been weather modification by seeding convective clouds with salt earlier in the day in the vicinity of the storm system that caused the flood. After the scheduled conference there was a discussion of a report on the relationship, if any, between the weather modification activity and the flood. The report, written by three scientific and legal specialists, submitted to Governor Richard Kneip and the Weather Control Commission of South Dakota, reaffirmed previous conclusions that the seeding did not contribute materially to the flooding. Had none been conducted, damage would have been the same. Another conclusion of the report was that regulation of all weather modification activities in the state is needed to preclude even the appearance of hazardous activity.

Operating programmes must have available information sources that will permit a safe shutdown of operations and a clearly stated procedure for so doing in the event of a flood threat, regardless of whether or not seeding could contribute to the hazard.

Although various topics were discussed, most of the conference concerned the modification of clouds to augment precipitation. Discussion of the modification of convective clouds produced proponents of both views of the success of precipitation. Papers were presented reporting both failure and success in certain conditions. Dr R. I. Sax (US Air Weather Service) showed that an attempt in 1971 to alleviate a widespread drought of severe proportions in Texas could claim no success, and that within present knowledge cloud seeding is not an efficient measure for relieving large scale droughts once such droughts are in progress.

On the positive side, Professor L. O. Grant (Colorado State University) presented the results of randomized seeding of summertime clouds over a portion of the Rocky Mountains. He reported a slight overall decrease of precipitation on seeded days, but found that when the meteorological conditions of the seeded days are categorized, there is evidence that precipitation may have been strongly affected in certain conditions to produce positive effects, and negative effects in differing conditions.

The conclusions to be drawn here are that when the field results of randomized seeding programmes have been investigated, and the results stratified to identify the type of meteorological conditions of augmented precipitation, then carefully controlled cloud seeding may produce beneficial increases in rainfall. Coupled with some of the experiments are investigations of these conditions with computer-simulated models, which, by variation of the parameter inputs into the models, are leading to a better understanding of why certain clouds respond to treatment for the augmentation of precipitation.

ACTINOMYCETALES

Actinos and Bifids

from a Correspondent

THE characteristics and practical importance of actinomycetes were central topics of the annual symposium of the Society for Applied Bacteriology held from July 10 to 13 at Loughborough University of Technology. Perhaps for the first time at meetings devoted to these organisms consideration of their antibiotic producing activities and their taxonomy was not allowed to dominate the meeting.

Dr W. B. Turner (ICI Ltd, Alderley Park, Macclesfield) reviewed the secondary metabolites of actinomycetes formed by way of a variety of pathways but included pigmented and odoriferous compounds as well as antibiotics. Dr T. Cross (University of Bradford) and Dr M. Goodfellow (University of Newcastle upon Tyne) presented a classification of the order into ten families which now include obligate symbionts of non-legumes (Frankiaceae), acid-fast bacilli which may be highly pathogenic (Mycobacteriaceae) and aquatic zoospore forming genera (*Actinoplanes*) as well as the more familiar streptomycetes. They limited the endospore producing species to the single genus *Thermoactinomyces* and defined the Nocardiaceae using presence of nocardomycolic acids and fragmentation as principal characters.

Studies of fine structure have enabled Dr S. T. Williams, G. P. Sharples and R. M. Bradshaw (University of Liverpool) to propose three basic patterns for spore formation in the order. They argued their case by including electron micrographs of endogenous spore formation in *Pranomonospora* and contrasted this with the septation mechanism in sheathed and unsheathed hyphae typical of *Streptomyces* and *Micromonospora* respectively. Professor D. A. Hopwood (John Innes Institute, Norwich) discussed genetic recombination in various genera; he also described his recent findings on the fertility system of *Streptomyces coelicolor* and the initial results of trans-

formation studies using the thermophilic endospore producing species *Thermoactinomyces vulgaris*.

Interesting aspects of streptomycetes as pathogens were unfolded by Dr D. A. Lapwood (Rothamsted Experimental Station, Harpenden) who dealt with the infection of potato roots and tubers by *Streptomyces scabies*, and the importance of soil moisture at the infection stage. Dr W. Blyth (University of Edinburgh) discussed the reasons for the high incidence of farmer's lung in certain areas of Scotland and suggested that the antigens of germinating spores might be implicated in this extrinsic allergic alveolitis caused by the inhalation of high numbers of thermophilic actinomycete spores.

Aspects of actinomycete ecology in soil and composts were discussed by Dr J. Lacey (Rothamsted Experimental Station), and Dr N. P. Burman (Metropolitan Water Board, London) reported on the presence and growth of actinomycetes in water treatment and distribution systems. Progress in understanding of the role of actinomycetes in nature and the possibilities of international cooperative projects in taxonomy were summarized by Professor D. Gottlieb (University of Illinois).

A very useful afternoon of the symposium was devoted to the anaerobic and microaerophilic actinomycete genera, so enabling dental, medical, soil and pollution microbiologists to discuss common problems. Drs G. H. Bowden and J. M. Hardie (London Hospital Medical College) spoke on commensal

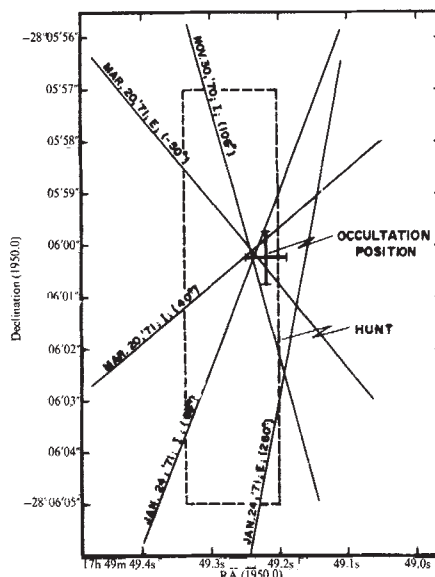
Position of the Pulsar PSR 1749-28

A GREAT increase in the accuracy of the known position of the pulsar PSR 1749-28 has resulted from observations of lunar occultations of the source carried out at Ooty. Writing in next Monday's *Nature Physical Science* (July 31) A. Pramesh Rao and S. Krishnamohan describe these observations, carried out on November 30, 1970, January 24, 1971, and March 20, 1971, and give the best position for the pulsar now available (see figure).

Altogether, five occultations were used to derive the pulsar position. The Ooty radio telescope (see Swarup *et al.*, *Nature Physical Science*, **230**, 185; 1971) was used in total power mode for all the observations, and in phase switched mode as well on March 30, 1971, with a bandwidth of 4 MHz and time constants of 22 ms and 50 ms for these two modes, respectively.

The position which Rao and Krishnamohan give for PSR 1749-28 is RA 17 h 49 m 49.22 s $\pm$ 0.03 s, Dec $-28^{\circ} 06' 00.2'' \pm 0.5''$; the accuracy of this position emphasizes the value of lunar occultations—it is only unfortunate that

the Moon does not travel in a convenient orbit for studying all pulsars in this way, and that in this particular case no optical object can be seen within 10 arc sec of the pulsar.



and pathogenic *Actinomyces* species in man and described serological methods developed for species and strain identification. Studies on gastroenteritis in breast fed infants by Dr A. T. Willis, Catherine L. Bullen and Kathleen Williams (Luton and Dunstable Hospital, Luton) illustrated the importance of *Bifidobacteria* in the gut at an early age. A rapid method for identifying *Bifidobacteria* species and therefore analysing different gut populations was described by Drs M. Catteau and F. Poncelet and Professor M. Beerens (CERTIA, Lille).

PLANT BIOCHEMISTRY

Nitrogen Metabolism

from a Correspondent

THE IUB/IUBS symposium on nitrogen metabolism in plants, held at the University of Leeds from July 10 to 14, showed the great progress that has been made recently in understanding the processes of nucleic acid and protein synthesis in plants.

Drs A. B. Legocki (College of Agriculture, Poznan) and J. E. Allende (University of Santiago) discussed polypeptide chain elongation on plant 80S ribosomes. They have isolated two elongation factors; EF1 is the factor responsible for promoting the binding of amino-acyl-tRNA to the ribosome and EF2, the translocation factor, is necessary for the movement of peptidyl-tRNA from the amino-acyl-tRNA site to the peptidyl-tRNA site on the ribosome. Both elongation factors require GTP for their action, and there is evidence that EF1 changes its configuration by loss of a subunit on binding with amino-acyl-tRNA. Dr A. Marcus (Institute for Cancer Research, Philadelphia) discussed the initiation of protein synthesis. Using tobacco mosaic virus RNA as a natural messenger in an *in vitro* system from wheat embryos, he has found that initiation proceeds as follows. The messenger RNA binds with the 40S subunit of the ribosome; initiating methionyl-tRNA then binds to the peptidyl-tRNA site on the 40S subunit and, finally, the initiation complex is completed by addition of the 60S subunit of the ribosome. The process requires ATP, GTP and two initiation factors. It is clear from these studies that although the mechanism of protein synthesis in plants is similar to that in bacteria, there are nevertheless important differences between the two synthesizing systems.

It is now accepted that, although plastids and mitochondria contain their own DNA and protein synthesizing systems, they are not completely autonomous. Nevertheless, as Drs R. J. Ellis and M. R. Hartley (University of Warwick) showed, it is possible to

obtain isolated chloroplast preparations which will bring about light-dependent synthesis of protein and RNA. The bulk of the protein made by such a chloroplast preparation consists of the large subunit of fraction I protein, although several other proteins, including at least one membrane-bound protein, are also synthesized. The product of RNA synthesis in the isolated chloroplasts is a large precursor of chloroplast ribosomal RNA (molecular weight 3.0×10^6). It has not yet been possible to demonstrate *in vitro* the processing of this precursor to form the ribosomal RNA.

The interactions of nuclear and plastid genomes in controlling plastid development are very complex. Professor L. Bogorad (Harvard University) has isolated nine mutants of *Chlamydomonas* which exhibit changes or deficiencies in chloroplast ribosomal proteins. Eight of these mutations may be assigned to nuclear genes, whereas the ninth is inherited uniparentally, suggesting a location in the plastid genome. Professor R. Hagemann (University of Halle-Wittenberg) has studied a mutant of *Pelargonium* which lacks plastid ribosomal RNA. The

mutation, which is located in the plastid genome, results in the formation of very small plastids which contain no chlorophyll, internal membranes or ribosomes. The plastids, however, do have a complete double outer membrane, are able to replicate, and therefore presumably to synthesize DNA. Evidently the proteins necessary for these latter features of plastid growth are not made on plastid ribosomes.

The view that plant hormones act directly on the genome is not now generally held. Nevertheless, plant hormones are obviously involved in the regulation of nucleic acid and protein synthesis *in vivo*. Professor F. Skoog (University of Wisconsin) suggested possible modifications of tRNA caused by cytokinins, and Dr L. Dure (University of Georgia) showed that abscisic acid is involved in preventing the precocious germination of cotton seed embryos, and that this action is dependent on RNA synthesis. Before such effects are understood, it is necessary to carry out biophysical studies of the rapid short-term effects of plant hormones, as was suggested by Dr R. L. Rayle (California State University, San Diego).

Viral Messengers

THE messenger RNAs of eukaryotic cells have long half-lives so that it has been suggested that control of the rate of synthesis of particular proteins may occur at the level of translation rather than transcription. Finding suitable experimental systems to test this idea has, however, proved difficult, which is one reason why the work reported by Perlman, Hirsch and Penman in *Nature New Biology* next Wednesday (August 2) will be welcomed.

Late during the infection of HeLa cells by adenoviruses almost all proteins made are viral. In such cells the polysomes are much smaller than in uninfected HeLa cells even though adenovirus proteins are on average at least as large as cell proteins. One explanation of this discrepancy is that the rate of initiation of translation of the viral mRNAs is limiting and much slower than the rate of initiation of translation of cell messengers so that the viral messengers are less densely packed with ribosomes than cell polysomes.

This idea is supported by the fact that when the rate of chain elongation is slowed some five-fold, by adding small amounts of cycloheximide, the size of viral polysomes in infected cells increases. In these circumstances chain elongation rather than initiation is presumably limiting. Moreover, the finding that the ratio of the different adenovirus proteins made in the presence of cycloheximide differs from the

ratio of proteins made in its absence suggests that adenovirus messengers are translated at different rates. That is the conclusion which Perlman *et al.* draw from comparisons of the pattern of synthesis of adenovirus proteins, in the presence and absence of cycloheximide, in HeLa cells cultured at 37° C and 40° C. And because initiation of translation in HeLa cells is inhibited at elevated temperatures it seems likely that any differences in the rate of translation of adenovirus messengers in these cells stem from differences in the rate of chain initiation.

Many viral messengers, like their cellular counterparts, terminate in a 3' sequence of some 50–200 adenylic acid residues. Whatever the role *in vivo* of these poly(A) tails they provide a convenient handle for fishing out messengers, and, as Zassenhaus and Kates report in the same issue of *Nature New Biology*, they can be exploited to achieve the efficient synthesis of complementary DNA by reverse transcriptase. The trick, of course, is to add a short oligomeric dT primer. This primer hydrogen bonds to the 3' poly(A) sequence of the messenger RNA template and provides reverse transcriptase with a free 3' hydroxy group on which to initiate synthesis of complementary DNA. Zassenhaus and Kates chose vaccinia virus mRNA made *in vitro* as a model system but their findings should be of general application.

COURTSHIP

Electric Attraction

from our Animal Behaviour Correspondent

THE electric gymnotid fish of Central and South America are known to use electricity to locate objects and also to communicate with each other in aggressive encounters; they generate electric signals from the muscles of the hind end of the body. According to C. D. Hopkins of Rockefeller University electric signalling is also used in courtship by the gymnotid *Sternopygus macrurus* (*Science*, 176, 1035; 1972).

Sternopygus macrurus, a common fish in mountain streams, is the first known example of a fish with sexually different electric discharges. Hopkins used wire electrodes amplified with a portable audio amplifier to detect the signals of this fish in the Rupunum District of Guyana. He found that the average male discharge was at 66.8 Hz, whereas that of the females was at 92.3 Hz, and that there was no overlap at all between the sexes. Most interestingly, he was able to show that the fish themselves are sensitive to this difference in discharge frequency.

Males apparently vary their signals during courtship, raising the discharge frequency or interrupting their signalling, but do not do this to other males or to females of other species. Hopkins played pure sine waves of different frequencies through copper electrodes to male *Sternopygus* and found that the fish gave courtship responses only to those frequencies typical of females of their own species. Not only can these fish distinguish their own species from other electric fish living in the same streams, they can tell a conspecific female from a male solely by the frequency of its discharge.

BIOLUMINESCENCE

Glow of Jellyfish

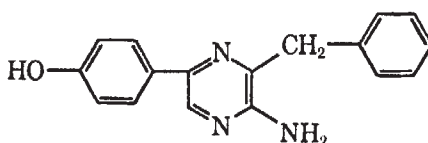
from a Correspondent

FOR several years O. Shimomura, F. H. Johnson and their colleagues at Princeton have been revealing the properties of the bioluminescent protein, aequorin, which is found in the jellyfish *Aequorea*, and they have recently published the structure of the light emitting moiety (*Biochemistry*, 11, 1602; 1972).

Most bioluminescent systems are of the so-called luciferase-luciferin type where the amount of light produced is proportional to the amount of a diffusible organic substance luciferin whose oxidation—catalysed by an enzyme luciferase—produces bioluminescence. In aequorin, however (and so far in the bioluminescent systems of only two other species, a sea shrimp and a paddle worm), the amount of light is proportional to the amount of protein.

Aequorin, with a molecular weight about 30,000, fluoresces normally only until calcium or strontium ions are added. Then it emits a strong blue luminescence and a highly fluorescent complex of aequorin:3Ca is irreversibly formed. The fluorescence spectrum of this product is the same as the spectral distribution of the light emitted during the reaction with calcium. The emission is so sensitive to calcium that aequorin may be used as a sensitive assay for calcium in biological systems.

The light emitting moiety, named AF-350 because of its ultraviolet absorption maximum at 350 nm, can be removed from aequorin by treatment with urea and cysteine, and extraction with butanol. Shimomura and Johnson used 45,000 jellyfish weighing 2 tons to give a pure sample of 125 mg of aequorin;



from this 1 mg of pure AF-350 was obtained. Studies on the purified AF-350 using ultraviolet, nuclear magnetic resonance, infrared, mass spectra and chemical tests indicate that it is most likely to be 2-amino-3-benzyl-5-(p-hydroxyphenyl)pyrazine. This structure was confirmed with model compounds and it seems that the AF-350 moiety is

probably bound to the protein by the amidine part of the pyrazine ring. It remains to be seen what intramolecular process produces the greater than 60 Kcal required for light emission.

At present there is no commercial source of aequorin and, as a help to all workers interested in using it to assay for calcium, Johnson and Shimomura have also published (*Nature New Biology*, 237, 287; 1972) a relatively simple method of preparation.

SUBCELLULAR STRUCTURES

Assembling Units

from a Correspondent

THE generation of subcellular structures was the topic of the first John Innes symposium at the John Innes Institute, Norwich, from July 4 to 6. In spite of the wide spread of subjects—which ranged from simple plant viruses to mitochondria—covered under this title, there was a good measure of cohesion at the meeting. Throughout the proceedings the emphasis was on the assembly of the structures discussed, up a rising scale of complexity.

The processes which are self-assembling from a few components and which can be studied *in vitro* are the most fully understood. Since the pioneering work of H. Fraenkel-Conrat and R. C. Williams, tobacco mosaic virus has been

Fusion of Tumour and Host Cells

OVER the past several years Klein and Harris and their colleagues have collaborated in investigations of the properties of hybrid cells obtained by fusing *in vitro* various malignant and non-malignant cultivated cells in an attempt to get to the bottom of the cytogenetics of malignancy, and in *Nature New Biology* next Wednesday (August 2) they revive the old idea that cell fusion may be one of the ways by which malignant cells arise *in vivo*.

The A9 mouse cell line is not highly malignant but if enough cells are inoculated into syngeneic or semi-allogeneic mice progressive tumours develop in a minority of the recipients. Harris and Klein explanted one such tumour and cultivated the cells *in vitro*, testing them for malignancy at each passage until they obtained a derivative line A9HT (high tumour incidence) which was much more malignant than the parental A9 line.

Both A9 cells and A9HT cells lack the enzyme inosinic acid pyrophosphorylase and cannot, therefore, grow in HAT medium containing aminopterin. When, however, tumours obtained by inoculating A9HT cells into suitable host mice are explanted into HAT medium a few of the cells sur-

vive and by 8–14 days have given rise to clones of HAT resistant cells which presumably contain inosinic acid pyrophosphorylase.

What is the origin of these HAT resistant tumour cells? Their modal chromosome number is 90–93 compared with the modal number of 53 of A9HT and 57 of A9. Moreover, the HAT resistant cells carry the H2 isoantigen complex of the host mice in which they were grown, in addition to the H2 antigens of the A9 line, and when the A9HT cells are passaged in mice carrying a translocation chromosomal marker the HAT resistant cells also have the marker chromosome.

There seems no escaping the conclusion that the HAT resistant cells arise by the fusion of the inoculated A9HT cells with normal host cells, and a series of control experiments indicate that the fusion event occurs *in vivo* rather than during the cultivation of the explants *in vitro* in HAT medium. As Klein and Harris and their colleagues say, fusion could provide the basis for genetic variation in cell populations, but whether in fact fusion is important in the aetiology of cancers can be decided only after investigation of the chromosomes of tumour cells.

a favourite example for such study and it has been used by Dr J. G. Atabekov (Moscow State University). Dr Atabekov—who was unable to be present at the meeting—has isolated fragments of the viral RNA and has found that fragments from the 5' hydroxyl end have a greatly enhanced ability to re-assemble with the protein. These results confirm the earlier work of P. J. G. Butler and A. Klug (MRC Laboratory of Molecular Biology, Cambridge).

A more complex system, and one where such detailed recognition of the viral RNA does not seem to occur, is found in cowpea chlorotic mottle and brome mosaic viruses. The RNA of these viruses occurs in three physically distinct but very similar types of particle, each of which is encapsidated with the same viral coat protein. Using this natural cleavage of the viral genome, Dr J. B. Bancroft (John Innes Institute) has obtained a hybrid virus, with part of its genome derived from each of the parent viruses. He has also been isolating mutants of some of the viral functions and, by the use of such mixed genome viruses, he hopes to map these mutations in spite of the lack of *in vivo* complementation which has proved so fruitful for such studies on bacteriophage.

A surprising result was reported by Dr A. M. Glauert (Strangeways Research Laboratory, Cambridge) who has investigated a protein component on the outer surface membrane of Gram-negative bacteria. After either freeze etching or negative staining these protein subunits are seen in the electron microscope to form a regular lattice which covers the maximum possible area of the surface. The subunits are released from the surface membrane, or even directly from the cell wall, by sonication and they will then reassemble to form a lattice which is very similar to the original one on the cell surface. This reassembly appears to take place at the air/water interface of a drop of the solution and without requiring any template or other information which is not within the protein subunits themselves. It is thus an example of a self-assembling system, and the main interaction with the other components of the membrane is presumably to require them as a surface upon which to assemble.

A more complicated assembly process occurs in the formation of bacterial flagella, where the rapid repolymerization of isolated flagellin, under mild conditions, has previously been shown by S. Asakura (Nagoya University, Japan) to require seed fragments derived from flagella filaments. Dr D. Kerridge (University of Cambridge) reviewed the structure of the bacterial flagellum and described how the filament is attached to the cell through a "hook" region and

a basal body and growth occurs only at the distal end of the filament. He also showed that the nature of the filament is determined by the protein from which it is assembled, with some mutant proteins forming only straight filaments, even when polymerized onto the ends of seeds from the normal helical filaments. The detailed structure of the filament was described by Dr A. Klug and there followed a discussion of how the transfer of energy from the base along the length of the filament, for the beating of the flagellum, could be an inherent property of the structure and not require any physical transport to accompany it.

The assembly of bacterial ribosomes (S. Fahnestock, University of Wisconsin) and of bacteriophage (W. B. Wood, California Institute of Technology, and E. Kellenberger, Mikrobiologie Biozentrum, Basle) have each filled many whole meetings and were only discussed very briefly. The possibility of *in vitro* complementation for defective mutants in the assembly of bacteriophage T4 has provided much knowledge of the sequence of events in this process and there are the interesting demonstrations of proteins which are not incorporated into the final virus (for example, the product of gene 38) or which are cleaved during the maturation process (for example, the product of gene 23).

For an organelle as complex and autonomous as the mitochondrion, the prospect of understanding the assembly is still distant. Dr B. Afzelius (University of Stockholm) described the structures which have been observed during mitochondrial growth and duplication and showed how these events have similarities to cell replication. Taking advantage of these processes to study DNA synthesis, Dr A. C. Arnberg (University of Gröningen and Wallen-

berg Laboratory, Uppsala) has shown the generation of a "D loop" by the synthesis of a single DNA strand, of about 500 nucleotides, complementary to one of the parental strands. At this point synthesis is checked and many of these structures are found in the DNA from a normal population of mitochondria. When DNA replication occurs, the synthesis of the same single strand resumes and synthesis of the complementary strand does not start until about 60 per cent of the DNA length has been copied. This second strand is then synthesized along the displaced parental strand, leading to the familiar semi-conservative replication.

NUTRITION

Diet and Heart Disease

from a Correspondent

CARDIOVASCULAR disease is almost certainly of multifactorial aetiology, and the Nutrition Society held a symposium on July 5 and 6 at the University of Surrey on the subject of diet as a risk factor in the disease.

Dr A. G. Shaper (London School of Hygiene and Tropical Medicine) drew attention to the finding between different countries that the severity of atherosclerosis seems to be related to serum cholesterol concentration, and to the high proportions of dietary energy derived from fat, but that is not necessarily true for individuals within any one country. Epidemiological studies suggest that hypertension is almost as powerful as the serum cholesterol concentration as a predictor of susceptibility to heart disease. Some workers had suggested that an increased concentration of serum triglyceride, independent of cholesterol, is also involved,

A Choice of Disulphide Pairings

IN next Wednesday's *Nature New Biology* (August 2) McKenzie and Shaw describe a situation in which two alternative disulphide pairings exist in a single protein molecule. In bovine β -lactoglobulin B, which is made up of a single species of chain, containing 162 residues, there is a single thiol group and two disulphide bonds per subunit. The thiol occurs in a part of the chain that contains two half-cystines (at positions 68 and 70) in a sequence ala-cys-glu-cys-leu. One of these is implicated in a disulphide bridge with cys-57 at the other end. Thiol labelling with radioactive iodoacetamide proceeds satisfactorily only in 8 M urea.

Subsequent peptide mapping reveals that the total counts taken up are distributed equally between two residues identified as cys-68 and cys-70. The

same result was obtained for other genetic forms of the protein. The question then is whether the two alternative disulphide pairings pre-exist in the molecule, or are the result of disulphide exchange during the period of some minutes in which it is exposed to the 8 M urea. The first alternative seems much the more probable, for the labelling ratio of the two positions was unaffected by the length of reaction or changes in the reaction pH, which would be expected to alter the extent of any exchange process.

It therefore seems that here is a case in which two disulphide pairings have comparable energetic probabilities. McKenzie and Shaw point out that such a situation could easily be overlooked in other proteins if specific experimental precautions are not taken.

HYDROBIOLOGY

New Committee

THE Institute of Biology, following the findings of a working party on the role of the biologist in the management of water resources (see *Biologist*, 18, 59; 1971), has set up a Standing Committee on Biologists in the Water Industry which will advise the council of the institute on the representation of the views of biologists working in these fields. The committee will further advise biologists on the part they can play in water management, the type of training best suited to the job and will hope to provide the stimulus for ensuring that the necessary applied research and development is undertaken. Furthermore, it is hoped that the committee will be able to produce a register of hydrobiologists working in Britain. Interested biologists are asked to contact Dr C. M. R. Pastakia, Briant & Harman, 16 Southwark Street, London SE1.

but this seemed unlikely. With reference to sucrose, Dr Shaper suggested that this is not a primary factor, but it acts synergistically with increased serum cholesterol and triglyceride concentrations.

Dr G. A. Gresham (University of Cambridge) emphasized that atherosclerosis is a human disease, and that attempts to reproduce it in experimental animals resulted in the development of only early facets of the disease process. Dr Gresham described a sequence of changes from the early fatty streak, through the development of a fibrous plaque to the complicated fully developed lesion. Early fatty streaks had been found in the large vessels of still-born babies so that it is possible that the development of the disease is a life-long process.

Professor P. Harris (Institute of Cardiology, London) gave a brief account of the structure of the myocardium as revealed by light and electron microscopy, and discussed the way in which energy, produced by the hydrolysis of ATP, is linked with the action potential, and the movement of sodium ions into the cells.

Dr M. C. Stone (Leigh Infirmary, Lancashire) discussed the role of diet in the management of hyperlipoproteinaemias. He and his collaborators have developed a simple method for studying the serum lipoprotein pattern by a combination of membrane filtration and nephelometry. Some types of hyperlipoproteinaemia, which may be indicative of a susceptibility to heart disease,

have been successfully treated by diets containing 50 g of fat and 200 mg of cholesterol. A substantial proportion of the fat which is given is polyunsaturated.

Professor F. Fidanza (Istituto Scienza Alimentazione, Perugia) described the epidemiological evidence for the fat theory of the aetiology of heart disease. Published records show a correlation between dietary fat and the incidence of arteriosclerosis. No such correlation is found in some peoples, such as the Masai and the Alaskan eskimos, and they are probably genetically adapted to such a diet. Professor Fidanza said that prospective epidemiological studies could give information that could be applied to individuals. He then discussed his own study on men aged 40 to 59 which had shown a significant correlation between the serum cholesterol concentration and the incidence of coronary heart disease, whereas energy consumption and the percentage of energy derived from protein or from fats showed no such correlation. There was, however, a variation from 3 to 29 in the percentage of energy derived from saturated fatty acids, and a high proportion was significantly correlated with coronary heart disease.

Dr A. J. Vergroesen (Unilever, Vlaardingen) has found that lauric and myristic acids increase the serum cholesterol concentration more than oleic acid, but that oleic acid acts synergistically with dietary cholesterol to produce high serum concentrations.

Professor J. Yudkin (Queen Elizabeth College, London) said that epidemiological evidence must be substantiated by experiments with animals and ultimately

with humans. He has found that sucrose diets produce changes in lipid metabolism and platelet stickiness in man and animals which would indicate that sucrose is a dietary factor in heart disease.

Professor H. Keen (Guy's Hospital Medical School, London) presented data showing the fairly high incidence of undiagnosed diabetes in a population study in Bedford. He discussed the relationship between diabetes and atherosclerosis in Western countries. The poor correlation found in Japan was of interest especially in view of the changes in diet now occurring there.

Dr Margaret D. Crawford (London School of Hygiene and Tropical Medicine, London) discussed the hardness of water and cardiovascular disease. A higher incidence of all forms of heart disease is found in areas where the rainfall is high and the water soft. Hardening the water supply can reduce deaths from cardiovascular disease. In soft water areas, there is more disease of the arteries, and it may be that the electrolyte balance in heart muscle is affected. Dr Crawford also pointed out that the trace elements chromium, vanadium and selenium have a protective effect probably by reason of their effects on lipid metabolism, whereas cobalt and lead are deleterious.

After discussing previous work that had shown that suitably motivated individuals will accept changes in diet, Professor M. J. Karvonen (Institute of Occupational Health, Helsinki) presented results of a study in Helsinki into the effect of changes on the ratio of dietary polyunsaturated to saturated fatty acids on serum cholesterol.

Chick Red Cells make RNA

In *Nature New Biology* next Wednesday (August 2) Madgwick, Maclean and Baynes report a set of quite simple and straightforward experiments which lead them to the conclusion that chicken erythrocytes actively synthesize RNA; these nucleated red blood cells are not apparently as metabolically inert as biologists have assumed them to be.

Madgwick *et al.* incubated either washed chick erythrocytes or unwashed whole blood collected in heparin with one or other of three labels, ^3H -uridine, ^3H -adenine and ^3H -thymidine. The last label was not incorporated to any significant extent which indicates, as expected, that the erythrocytes do not make much DNA. By contrast, both ^3H -uridine and ^3H -adenine are incorporated into a TCA precipitable material by a reaction that is sensitive to inhibition by actinomycin D.

The results of a variety of tests indicate that these two labels are being incorporated into RNA, and microscopic

examination of stained smears indicates that the incorporation is not restricted to reticulocytes. The nature and role of the RNA made in these circulating and mature chick erythrocytes have yet to be elucidated, but it is not without significance that in some experiments the inhibition of RNA synthesis by actinomycin D was accompanied by an inhibition of incorporation of ^3H -leucine.

The life span of chick red blood cells in the circulation is about 28 days, much shorter than the life span of amphibian red cells, which is measured in hundreds of days, and this may explain why chick but not amphibian erythrocytes synthesize RNA; the chick cells may simply be comparatively immature. In any event, as Madgwick and his colleagues comment, anybody using nucleated erythrocytes to study the reactivation of nuclear metabolism would be well advised to use amphibian rather than chicken red cells.

Man-powered Flight as a Sport

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A successful future for man-powered flight may lie in its development as a sport.

MAN-POWERED flight is usually considered from one of two extremes—it is regarded either as an activity whose sole purpose is the winning of the Kremer prizes or as an impractical exercise where people try to emulate the birds by flapping wings attached to their arms. In practice, however, there have been important developments over the past decade, and we are now in a position to discuss the design of practical man-powered aircraft that would be suitable for sports flying.

It is now more than ten years since the first "true" man-powered flight took place when the Southampton University machine covered a distance of 50 yards from take-off. Since then, twelve aircraft of differing configurations and with wing spans ranging from 64 to 120 foot have achieved man-powered flights¹. The twelve man-powered aircraft are listed in Table 1.

None of these machines has complied with the requirements of the Kremer competitions which initially defined the performance requirements. There are several reasons for this, the chief of which is the increase of power required for the climb. All the aircraft listed in Table 1 would need to fly at an altitude of, say, 2 to 3 foot for most of the course in order to take

advantage of the ground effect, then climb back to the specified 10 foot at the finishing point. Power for climbing can be related to the cruising power and the angle of climb θ° by the simple relationship²

$$P_{\text{climb}} = P_{\text{cruise}} \left(1 + \left(\frac{L}{D} \right) \frac{\theta}{57.3} \right)$$

so that with an L/D (lift to drag) ratio of 50 or more, as achieved by the later aircraft, the climbing power would be approximately twice that for cruise with a 1° climbing angle.

In this respect the later £5,000 Kremer competition in which competitors have to complete two "slalom" runs³ is in reality probably more difficult than the £10,000 Kremer competition with its figure-of-eight course.

Man-powered Aircraft Development

It does not follow that the Kremer targets are unattainable, but there must be further developments before the prizes can be won. The competitions themselves do not, however, provide the necessary encouragement for these developments.

The problem is that all the aircraft have so far been designed for a direct attempt at the competition where the crew is required to provide all the power for the flight unaided by any help from the atmosphere. In order to extend flights the aim

Table 1 Data on the Twelve Man-powered Aircraft

	Span (foot)	Wing area (foot ²)	Aspect ratio	Empty weight (pound)	Flying weight (pound)	Wing loading pound foot ⁻²	Country	First flight
SUMPAC	80	300	21.3	128	269	0.90	Britain	1961
Puffin I	84	330	21.4	118	267	0.81	Britain	1961
Puffin II	93	390	22.0	140	290	0.78	Britain	1965
Linnet I	73	288	18.5	105	230	0.80	Japan	1965
Linnet II	73	280	19.0	99	225	0.80	Japan	1967
Linnet III	83	325	21.2	111	232	0.72	Japan	1970
Linnet IV	79	—	—	119	237	—	Japan	1971
Malliga aircraft	64	262	15.6	113	239	0.91	Austria	1967
Santa-Meada	73	290	17.9	122	245	0.85	Japan	1970
OX-Weybridge aircraft	120	480	30.0	125	276	0.57	Britain	1971
Jupiter	79	308	20.0	146	296	0.96	Britain	1972
Liverpuffin	64	305	13.3	140	300	0.98	Britain	1972

has been to reduce power input by increasing wing spans, so the resulting machines are subject to several restrictions. First, they can only be flown in very calm conditions, either at dusk or early in the morning, so only limited flying experience can be achieved. Second, they can only be entrusted to expert pilots, so the power available for propulsion is limited. Third, construction can only be attempted by large dedicated groups—the Weybridge aircraft, for example, involved about 10,000 man hours of construction time. Fourth, such machines require that large workshop/hangar and flying field facilities are readily available.

The last two restrictions obviously limit the number of such aircraft that can be built and the present difficulties within the aircraft industry mean that future attempts at building aircraft with wing spans of more than 120 foot—the size of the Weybridge or Toucan⁴ machines—cannot be envisaged.

These arguments suggest that the design and construction of man-powered aircraft solely for direct attempts at the Kremer competitions is an impractical exercise at the present time. There must, however, be some positive aim in any endeavour, and it therefore seems that man-powered flight should be developed as a sporting activity. This idea can be justified by the enthusiasm that would undoubtedly result from such an aim and also by the present wave of interest in hang gliding in the United States. Hang gliding is similar to man-powered flight in that it is a marginal aeronautical activity in terms of flight duration, but is far more dangerous because launches are made from hill tops whereas man-powered flights always take place close to the ground.

If competitions could be developed, they would provide a powerful incentive for improving the performance of the aircraft themselves, so the change of man-powered flight into a sporting activity could eventually lead to the winning of the Kremer prizes.

Technical Developments

The performance of man-powered aircraft is a function of the aircraft weight to power ratio (W/P). This ratio can be restated in terms of performance parameters in order to assess the relevant design criteria.

$$\frac{W}{P} \propto \frac{L}{D^{1/2} V} \propto \frac{C_L}{(C_{D0} + \frac{K}{\pi A} C_L^2) C_L^{-1/2}} \propto \frac{1}{\frac{C_{D0}}{C_L^{3/2}} + \frac{K}{\pi A} C_L^{1/2}}$$

where V is the aircraft velocity, C_{D0} is the overall profile drag coefficient, C_L the lift coefficient, K the induced drag factor due to the effect of the ground, and A is the aspect ratio. Obviously to improve the weight to power ratio the term $(C_{D0}/C_L^{3/2} + (K/\pi A)C_L^{1/2})$ needs to be decreased.

Graphs of the term $(C_{D0}/C_L^{3/2} + (K/\pi A)C_L^{1/2})$ against lift coefficient are presented in Fig. 1 for C_{D0} equal to 0.015 and $K/\pi A$ equal to 0.01, 0.015 and 0.02 respectively. This value of C_{D0} is entirely realistic for man-powered aircraft, whereas the values of $K/\pi A$ are equivalent to rectangular wings working within the ground effect region with aspect ratios of 20, 13^{1/3} and 10 respectively. Evidently most improvement in performance can be gained by an increase in working C_L within the range up to 1.2. For larger values of C_L the improvements are very much smaller, and with low aspect ratios are negligible.

The most important technical development of the past decade has been the improvement of aerofoil sections for use at the low Reynolds numbers appropriate to man-powered flight. Whereas the Southampton and Puffin I machines of 1961 were based on C_L values of 0.85 and 0.8 respectively, it is now possible to base the design of man-powered aircraft on the certain achievement of a value of C_L of 1.2 with a corresponding low aerofoil profile drag. This allows greater freedom for the designer and is the chief reason why compact man-

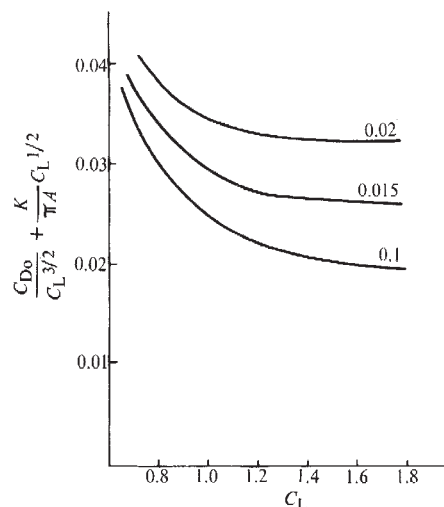


Fig. 1 Variation of aircraft weight/power parameter with lift coefficient.

powered aircraft suitable for sports flying can now be considered.

Experience with actual man-powered aircraft has indicated that fuselage design is not critical because the parasite drag is only a small part of the total aircraft drag. Care was exercised in the design of many of the man-powered aircraft to minimize fuselage drag by encasing the pilot in suitably streamlined fully enclosed cockpits. The Malliga aircraft, however, has the pilot pedalling in a reclining position with an open cockpit with a streamlined fairing behind his head (Fig. 2). In spite of increasing drag, open cockpits allow the pilot to pedal in more comfort and so in practice may result in an improved performance.



Fig. 2 The Malliga aircraft at Zeltweg, Austria.

Pedalling—either in a cycling position or in a reclining position—has proved to be a most suitable form of power input. Earlier it was considered that take-offs could be achieved using man-power alone only by having a driven undercarriage⁵. This has been disproved by the Linnet and Malliga aircraft which have take-off distances comparable with the other aircraft but utilize propeller thrust alone.

The pilot weight constitutes most of the total aircraft weight, so appreciable reductions in airframe weight are necessary to

make a worthwhile saving in the power requirements. From the sporting aspect, the important features are the robustness of the structure, whether the aircraft can be transported with ease, whether it will need repairing after each flight and whether it will stand a certain amount of mishandling both on the ground and in the air. In this respect two interesting developments over the past decade have been the Czerwinski laced joint⁶ for thin metal tubes and the use of expanded polystyrene for wing secondary structures¹. The Malliga aircraft utilizes a primary structure of aluminium tubing (diameter 4 inch) for the wing and 1 inch thick expanded polystyrene for the ribs with thinner sheet expanded polystyrene for the covering of the leading edge. This provides a robust structure that can be constructed in a relatively short time.

Thin aluminium tubes jointed by fibreglass tape and then coated with epoxy resin are used for the whole of the primary structure of the wing on the Weybridge machine. This has been loaded statically, and the measured deflexions are in good agreement with the estimated ones.

With regard to control of man-powered aircraft, the position is really no clearer than it was ten years ago. Most of the aircraft that have flown employ directional longitudinal and lateral control surfaces. SUMPAC incorporated an all-flying-tail plane that was difficult to control, so the conventional elevator used for Puffin and Linnet might seem to be a more suitable choice. With the increased wing span of Puffin II the rudder became ineffective because of the variation of induced drag on each wing tip during a turn. Fully controlled turns could only be executed by the provision of wing tip drag rudders which increased the drag of the lower wing tip. Directional control of the Toucan is by means of wing tip drag rudders alone.

Several systems for lateral control have been built. Conventional ailerons were used on SUMPAC and Puffin, and transverse wing tip ailerons are used on the Malliga aircraft; the Weybridge aircraft, on the other hand, incorporates a mechanism for varying the incidence of each wing half, a system that was originally used on the pre-war Haessler-Villinger machine. It is rumoured that Linnet III and IV were built without ailerons, but had the dihedral angle increased to 6% to maintain lateral stability. The feasibility of this has since been proved by Liverpoolpuffin which does not have any form of lateral control in order to simplify both the pilot's task and the wing construction.

Future of Man-powered Flight

From the design point of view, the initial requirements for man-powered flight as a sporting activity are four-fold. First, the aircraft should be simple and constructable by individuals or by small groups with limited workshop facilities. Second, it should be easily transportable to the flying field and capable of being rapidly assembled for flight. Third, it should be capable of being flown in light wind conditions of, say, 10 knots, otherwise flying would be very restricted. Fourth, it should be sufficiently robust to withstand the inevitable mishandling during transportation and during the training of the pilot.

All these requirements point to aircraft of minimum wing span compatible with the necessary low power input. At present the Malliga aircraft complies most closely with these requirements (Fig. 2), but it is now feasible to consider even smaller aircraft because suitable aerofoil sections with good values of C_L are now available. Fig. 3 shows a family of curves for man-powered aircraft of different wing spans; the curves are based on the use of the Wortmann FX-63137 aerofoil section working at a value of C_L 1.15. This aerofoil section has been fully tested in a wind tunnel, and the validity of the test results has been confirmed by the use of this section for Puffin II. It is assumed that the wing plan form is rectangular, that the fuselage has the same parasite drag as

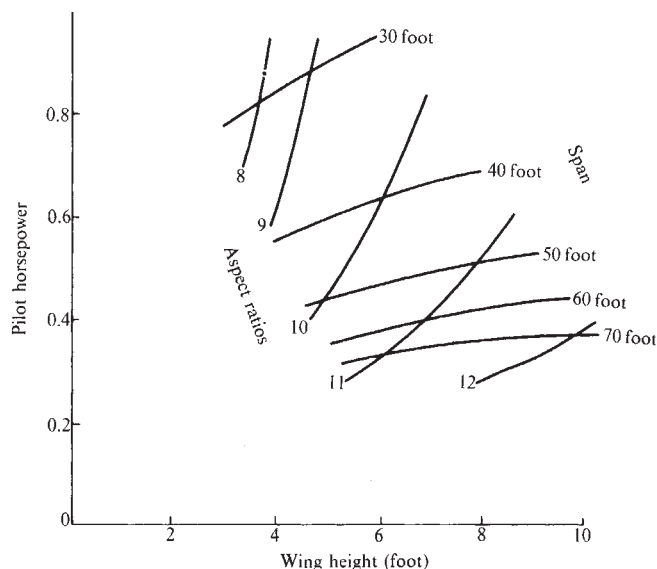


Fig. 3 Variation of minimum pilot input power with wing height.

the Southampton fuselage and that the wing is related to the wing span by the simple formula

$$W(\text{pound}) = 190 + \text{wing span (foot)}$$

A wing span of 50 foot would thus result in an aircraft weight of 240 pound.

It is therefore possible to consider a man-powered aircraft of span 50 foot that would require a power input of 0.48 horse power when flying at a wing height of 10 foot above the ground. Such an aircraft would comply more closely with the requirements of a sporting machine than any of the machines that have flown. Although probably not ideal, the construction of machines of this size would allow the initial development of man-powered flight as a sporting activity and could provide the knowledge for future design improvements.

During take-off the power requirement would be less than 0.48 horse power because of decrease in the induced drag, so that an average fit man whose weight was not greater than 150 pound could be sure of at least short flights of, say, 50 to 100 yards. Performance could be extended by the use of athletes as pilots or by utilizing low altitude convection up-currents. The former would be the obvious requirement for competition at national level and would be practicable because such a machine would be easier to control than any of the man-powered aircraft that have flown.

The utilization of up-currents would be the preferable method of increasing performance because many more pilots would be able to participate in sporting man-powered flight. Data on very low altitude up-currents are limited, but there is certainly evidence of useful lift above runways on warm sunny days. Rising air with a vertical velocity of 1 foot s^{-1} would be adequate for man-powered aircraft, and should theoretically be found 10 foot above a runway which is only 2° F warmer than the surrounding grass. Up-currents can, however, only be maintained by downward flows in adjacent regions, so a great deal has to be learnt before extended flights become a reality; this knowledge can only be gained by actual flights.

¹ Sherwin, K., *Man Powered Flight* (M.A.P., 1971).

² Spillman, J. J., *J. Roy. Aero. Soc.*, **66**, 699 (1962).

³ *Conditions for the £5,000 Kremer Competition* (Royal Aeronautical Society, 1967).

⁴ Pressnell, M. S., *J. Roy. Aero. Soc.* (in the press).

⁵ Shenstone, B. S., *J. Roy. Aero. Soc.*, **72**, 655 (1968).

⁶ Czerwinski, W., *J. Roy. Aero. Soc.*, **71**, 9 (1967).

Isolation, Identification and Synthesis of a Specific-behaviour-inducing Brain Peptide

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A pentadecapeptide isolated from the brain of rats trained to avoid the dark has been chemically identified and its structure confirmed by synthesis. It induces dark avoidance in untrained animals.

EXPERIMENTS were reported in 1968 in which dark avoidance was induced in mice treated with extracts of brain taken from rats that were trained to fear the dark¹. During the subsequent two years, brain material was collected from more than 4,000 trained rats and the dark avoidance-inducing substance was isolated. We now report the identification of this substance as a pentadecapeptide, the determination of the amino-acid sequence and its confirmation by synthesis.

Donor rats were trained and recipient mice tested as described before¹. The test consisted in recording automatically, by means of a photoelectric system, the time spent in the dark and lighted compartments of the apparatus. Before injection of the material to be tested, the mice were screened for dark preference and the only animals used were those that spent in the dark box at least 90 s out of the total of 180 s. Injections were done intraperitoneally into groups of six to twenty-four mice, randomly distributed under code numbers together with the controls, to keep the observers ignorant of the treatment received by the animals. The dose injected was equivalent to 1 g of fresh weight of brain. The animals were tested twice on the day following the injection and twice more the day after. The mean of the four values for each animal was used to calculate the mean of the group. Table 1 shows the results obtained with 115 extracts prepared from twenty to 100 trained rat brains. The thirty-four control extracts were made from untrained rat brains.

The same procedure was used for testing the fractions obtained in the successive steps of isolation and purification of the active material. These steps included extraction of RNA, dissociation, by dialysis at low pH, of the complex formed between the active peptide and RNA², gel filtration on 'Sephadex G-25' and thin layer chromatography (Fig. 1). The fractions obtained were lyophilized and injected to groups of mice, as described above. After gel filtration, the dose injected was increased to 2 g of fresh weight of brain, because of the loss of active material during purification.

Table 1 Mean Times spent in the Dark Box (DBT) by Mice before and after Injection of Extracts of Brain taken from Dark-Avoidance-Trained and Untrained Rats

	DBT	±	s.d.	No. of extracts
Before injection	130		15	149
After injection of trained extract	52 *		17	115
After injection of control extract	120		20	34

* Mean significantly different from the two others ($P < 0.001$ by t test).

Elution of the 'Sephadex' columns was monitored by ultra-violet absorption at 280 nm. Activity reached its peak at 480 ml. Angiotensin amide, used as a marker, eluted around 580 ml. When the active fraction obtained by gel filtration was further fractionated by thin layer chromatography, five ninhydrin-stained spots were obtained at R_F 0.36, 0.42, 0.55, 0.66 and 0.80 (Fig. 2). The spots were eluted with pyridine, acetic acid, water (1:10:289), lyophilized and tested for activity. A 3 mg sample of the active fraction obtained after gel filtration was placed on thin layer plates. The material eluted from each of the five spots was dissolved in 5 ml. of water. The eluate of the R_F 0.55 spot was active at 0.05 ml. per mouse, those of the other spots were inactive up to 0.5 ml., the highest dose tested.

These results indicated that at least 90% of the activity was concentrated in the R_F 0.55 spot. Attempts to resolve the active spot into further constituents were made by thin layer and paper chromatography with the following solvent systems: (a) n-butanol, acetic acid, pyridine, water (15:3:10:12); (b) ethanol, ammonia, water (7:1:2); (c) methanol, water (99:1); and (d) phenol, water (75:25). All the procedures yielded a single spot (R_F 0.44 in (a), 0.42 in (b), 0.65 in (c)); solvents (a) and (d) used on paper chromatography gave single spots (R_F 0.55 and 0.23 respectively). Amounts of 5–10 μ g of the material were used in each of the experiments. The solvent front was allowed to run up to 16–18 cm.

In view of the comparative insensitivity of the conventional chromatographic methods, the purity of the active material was further tested by the microdansylation method, based on the formation of fluorescent derivatives with 5-dimethylamino-1-naphthalenesulphonyl chloride. The technique described by Neuhoff *et al.*³ can detect pmol quantities of amino-acids and peptides. Samples of 20–200 ng of the dansyl derivative of active material were applied to polyamide plates (3 cm $\times$ 3 cm)

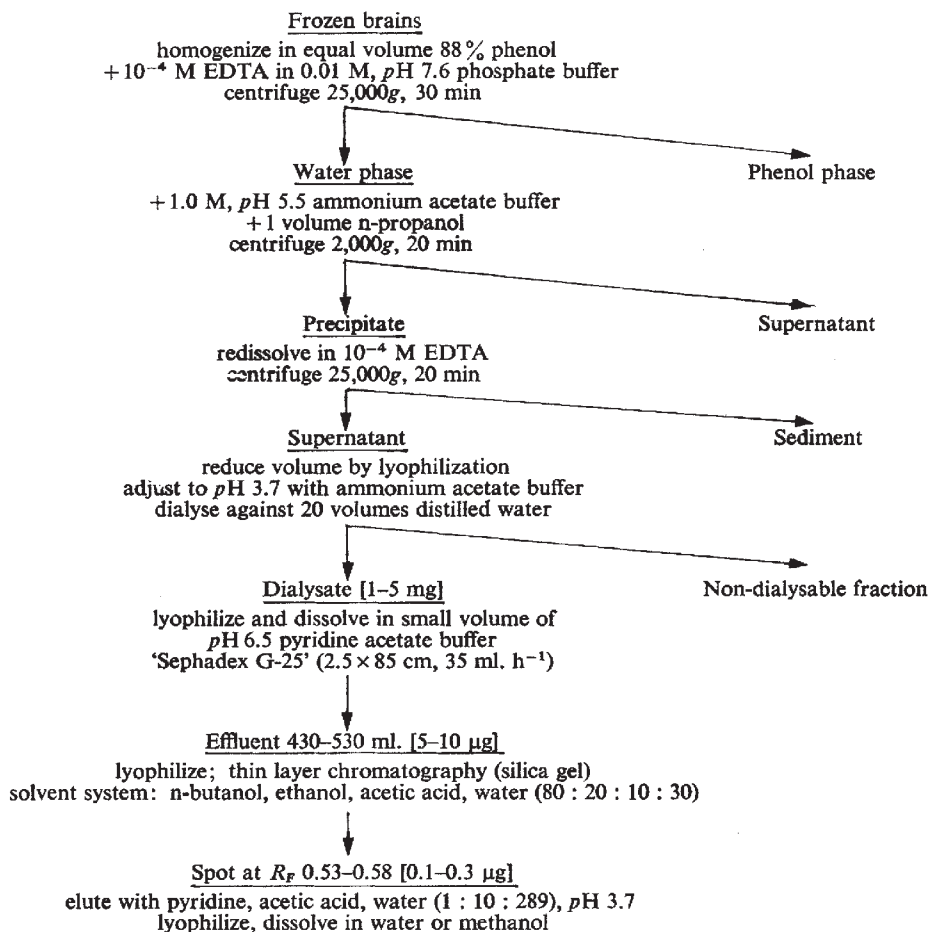


Fig. 1 Diagram of the purification of scotophobin. Active fractions underlined. Figures in square brackets indicate yield per g of fresh weight of brain. The phenol phase obtained at the initial step may yield some active material but purification was found too time-consuming to be practical. Further details in text.

and chromatographed in two dimensions with water, formic acid (100:1.5) and benzene, acetic acid (9:1). With less than 100 ng, the active material appeared as a single fluorescent spot at R_F 0.16 in both dimensions. At 100 ng or more, however, further spots appeared corresponding to amino-acids (Gly, Leu, Val) and two unidentified substances (R_F 0.86-0.33, 0.20-0.69). Most amino-acids are detectable in amounts of 0.05 and 0.1 ng, so the total amino-acid impurities could not represent more than a fraction of 1%. The unidentified spots may correspond to unusual amino-acids, amines or peptides.

In one experiment, amounts of 100 g of brain taken from trained and untrained rats were extracted identically by the procedure shown in Fig. 1. The thin layer chromatogram showed that the R_F 0.55 spot was absent from the untrained brain preparation, although the other spots had apparently the same size and intensity of colour (Fig. 2). The lowest limit of detection of the material contained in the R_F 0.55 spot is about 2 µg and the yield from 100 g of brain is between 10 and 30 µg. The untrained brain therefore contained less than 0.2 of the active material, if any. The absence of this material from untrained brain was further confirmed by the dansyl method which allows its detection at 50-100 ng. The amount of extract corresponding to 5 g of untrained brain failed to show the dansyl spot corresponding to the active material while 100 mg of trained brain gave consistently positive results.

A qualitative amino-acid analysis, by means of the micro-dansylation procedure, revealed the presence of the following amino-acids in the hydrolysate of the R_F 0.55 spot: Ala, Asp, Glu, Gly, Lys, Ser, Tyr. Dansylation followed by hydrolysis showed Ser as the *N*-terminal group. Having found previously

that the active substance was hydrolysed by trypsin, we incubated 50 µg of the material with 200 µg of crystalline trypsin at pH 8 in a volume of 40 µl. After 1 h at room temperature the mixture was dialysed at 4° C in a micro-dialyser⁴ against 30 ml. of water for 4 h. The dialysate was lyophilized and analysed by the standard thin layer method. Three spots appeared, one at R_F 0.26 (T_1), a second at R_F 0.40 (T_2) and a third at R_F 0.57, representing the unhydrolysed portion of the original material. After a hydrolysis and dansylation, T_1 yielded Asp, Glu, Gly, Lys and Ser, and T_2 Ala, Glu, Gly, Ser and Tyr. The *N*-terminal group of both fractions was found to be Ser. An unsuccessful attempt at determining the *C*-terminal group by carboxypeptidase A suggested that the group was blocked.

The combined results of these analyses indicated the following approximate structure: Ser-(Asp, Glu, Gly)-Lys-Ser-(Ala, Glu, Gly, Tyr)NH₂. A quantitative amino-acid analysis gave the following results (nmol µg⁻¹): Lys, 0.73; Asp, 2.12; Ser, 1.33; Glu, 2.90; Gly, 2.13; Ala, 0.65; Tyr, 0.81; and large amounts of ammonia. No other amino-acids were reported to be present in amounts above 20% of Asp. The accuracy of these figures is limited by the difficulty of weighing the amounts of material we had to handle. The total amount of purified substance obtained was slightly over 300 µg. This was dissolved in water and aliquots of this solution were taken for the tests described above. Since the accuracy of the absolute weight of the sample could not affect the proportion of amino-acids in the peptide, we assumed that it contained fifteen amino-acid residues: 4 Glx, 3 Asx, 3 Gly, 2 Ser, Ala, Lys, Tyr.

In view of the small amount of material left at this point

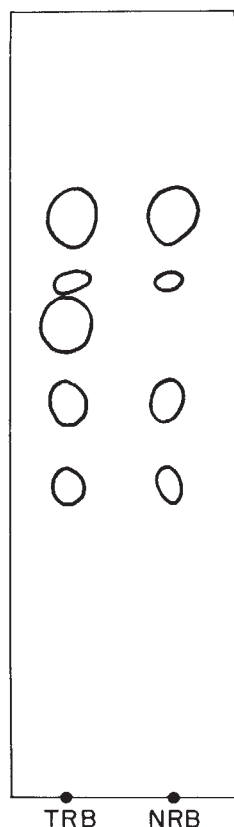


Fig. 2 Thin layer chromatography of brain preparations from dark avoidance trained (TRB) and untrained rats (NRB). The active fraction obtained by gel filtration was applied to silica gel plates. The solvent system was *n*-butanol, ethanol, acetic acid, water (8 : 20 : 10 : 30). The starting material was 100 g of brain in both preparations.

(130 μ g), the only method of sequential analysis available to us was mass spectrometry. Samples were first trifluoroacetylated to protect all free amino-groups and then treated with diazomethane to convert all free carboxyl groups to the corresponding methyl esters. The reagents were removed under vacuum and the sample introduced by means of a direct introduction probe into a CEC 21-110B (Lot 9) high resolution mass spectrometer. Ions were detected with an Ilford Q2 photoplate. The distances were measured on the photoplate with a Gaertner microdensitometer-linear comparator.

Samples of 20 μ g of the whole peptide and corresponding amounts of the tryptic fragments were used, each repeated twice. With this amount of material we could not expect to obtain the molecular ion of the whole peptide nor regular series of increasingly shorter sequences⁵. We found, instead, a large number of fragments produced either pyrolytically in the probe tip or by electron impact. The accurate masses of these fragments, calculated by computer, were examined manually for peptide sequences compatible with the approximate structure given above. The first tripeptide and the subsequent dipeptides overlap sufficiently to indicate the

tentative sequence (A) given in Fig. 3 (Table 2). A few other tripeptides were also found⁶, but they required the assumption of the loss of more than one group.

Positions 2, 5 and 11 of sequence A remained uncertain. The mass spectrometric data gave Asp, Glu and Glu, respectively, but the possibility of some of the amide groups being lost in the ionization process could not be excluded. In fact, the structure having the three free carboxyl groups, synthesized by Dr B. Weinstein at the University of Washington, was found to have only 2.5% of the activity (75 U mg^{-1}) of the natural product. We synthesized the fully amidated peptide (C) and found that it had 10% activity (300 U mg^{-1}). Finally, sequence D, with Asp in position 2 and Gln in 5 and 11, gave first 2,000 U mg^{-1} but after further purification on 'Sephadex LH-20' and thin layer chromatography, its activity reached that of the natural peptide (3,000 U mg^{-1})*.

The synthesis used the technique of Merrifield⁷ in a manner similar to that described by Bayer *et al.*⁸. *N*-tert.-butoxycarbonyl (Boc) amino-acids were prepared from tert.-butyl azide and the appropriate amino-acids as described by Schnabel⁹. The active esters used in the synthesis were prepared according to the procedure described by Bodansky^{10,11}. The synthesis was designed so that cleavage of the protected pentadecapeptide from the resin could be achieved by direct ammonolysis. For that purpose aspartic acid was attached to the growing peptide chain as 2-(*p*-di-phenyl)-isopropylloxycarbonyl(Dpoc)- β -tert.-butyl aspartate. The Dpoc protecting group could be selectively cleaved with acetic acid in the presence of the tert.-butyl ester as described by Sieber and Iselin¹².

Boc-O-benzyl-L-tyrosine was esterified to 800 mg of chloromethylated polymer ('Bio-Beads', S-X 2, 200–400 mesh, 0.7 mequiv. chlorine g^{-1}) in absolute ethanol for 48 h under reflux using triethylamine as acid binding reagent. A sample of the dried Boc-O-benzyl-tyrosyl-polymer was hydrolysed and the amino-acid analysis showed 0.1 mmol of tyrosine g^{-1} resin.

The following reaction cycles were used to build the amino-acid sequence from 15 to 3: cleavage of the Boc-groups by addition of 20 ml. of N HCl in glacial acetic acid and shaking for 30 min; washing three times with 20 ml. portions of glacial acetic acid, absolute ethanol, *N,N*-dimethylformamide (DMF) and chloroform for 10 min; washing three times with 20 ml. portions of chloroform and methylene chloride; coupling of the new amino-acid (*p*-nitrophenyl esters) to the free amino-groups on the polymer in DMF and methylene chloride. Ala, Ser, Lys were attached to the polymer by dicyclohexylcarbodiimide (DCC) coupling in methylene chloride. Aspartic acid in position 2 was introduced as Dpoc-O- β -tert.-butyl aspartate, which was obtained by treating the corresponding DCHA-salt with citric acid in methylene chloride. The Dpoc-group was split off by shaking the resin for 8 h with 30 ml. acetic acid¹² and finally the *N*-terminal amino-acid serine was attached as described above. The amino-acid side chains were protected as follows: serine and tyrosine, O-benzyl; lysine, *N*- ϵ -carbobenzoxy (Cbo) and aspartic acid, β -O-tert.-butyl. Boc-amino-acids, Boc-amino-acid *p*-nitrophenyl esters and Dpoc- β -O-tert.-butyl aspartate were used in a six-fold

* One unit of activity is the amount of material that reduces the mean time spent in the dark from 130 to 60 s. It is different from the unit previously used¹.

A	Ser-	Asx-	Asn-	Asn-	Glx-	Gln-	Gly-	Lys-	Ser-	Ala-	Glx-	Gln-	Gly-	Gly-	Tyr-	NH ₂
B	Ser-	Asp-	Asn-	Asn-	Glu-	Gln-	Gly-	Lys-	Ser-	Ala-	Glu-	Gln-	Gly-	Gly-	Tyr-	NH ₂
C	Ser-	Asn-	Asn-	Asn-	Gln-	Gln-	Gly-	Lys-	Ser-	Ala-	Gln-	Gln-	Gly-	Gly-	Tyr-	NH ₂
D	Ser-	Asp-	Asn-	Asn-	Gln-	Gln-	Gly-	Lys-	Ser-	Ala-	Gln-	Gln-	Gly-	Gly-	Tyr-	NH ₂
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	

Fig. 3 Determination of the sequence of scotophobin. A represents the tentative sequence deduced by mass spectrometry. In view of the uncertainty at positions 2, 5 and 11, sequences B, C and D were synthesized. The biological and chemical properties of sequence D are identical to those of natural scotophobin.

excess. The reaction time was 3 h in the case of the DCC method and 8 h in the case of the *p*-nitrophenyl esters. Excess reagents were removed by washing with methylene chloride, DMF, ethanol, acetic acid, ethanol and methylene chloride.

The dried, fully protected pentadecapeptide-polymer was transferred into a pressure bottle, suspended in 50 ml. DMF and 50 ml. liquid NH_3 was added at -72°C . The bottle was closed and the reaction mixture was shaken for 7 days. (A Parr medium pressure hydrogenation apparatus was used.) After release of the pressure the resin was filtered off, washed with DMF and the combined filtrate was evaporated *in vacuo*. The yield was about 100 mg.

The above pentadecapeptide amide was suspended in trifluoroacetic acid and anhydrous HBr (bromine free) was bubbled through the solution until saturation. Anisole was used as a scavenger. The reaction mixture was kept at room temperature for 2 h under a slow stream of HBr. After removal of the HBr and evaporation of the trifluoroacetic acid *in vacuo*, the crude product was dissolved in glacial acetic acid and precipitated with ether. The yield at this point was about 80 mg.

A portion of the crude product (55 mg) was dissolved in methanol and the alcoholic solution was submitted to gel filtration on 'Sephadex LH-20' using methanol for elution. The active fraction, determined by bioassay, weighed 42.8 mg. It was further purified by thin layer chromatography with our standard method. Three ninhydrin-positive spots at R_F 0.07, 0.38 and 0.57 were obtained. Elution of the spot at R_F 0.57 gave an overall yield of 39.6 mg. Amino-acid analysis (6 N HCl, 36 h, 115°C) gave the following molar ratios, Gly being taken as 3.00; Ala, 1.05; Gly, 3.00; Asp, 2.89; Glu, 3.85; Tyr, 1.05; Lys, 0.95; Ser, 1.90; NH_3 , 7.15. For further details of the synthesis, see Parr and Holzer¹³.

Fig. 4 shows the dose-response curve of the natural compound and synthetic peptide D. The lines were calculated by the least squares method. The dose reducing DBT to 60 s (unit activity) was 270 ng (95% confidence limits 186–396) for the natural product and 300 ng (238–377) for the synthetic substance. The slope functions were 2.34 (1.44–3.78) and 2.55 (1.50–4.30) respectively. The potency ratio, 1.11 (0.72–1.71), and the slope ratio, 1.09 (0.54–2.22), indicated that there was no significant difference between the behavioural activity of the two substances. All values were calculated by the method of Litchfield and Wilcoxon¹⁴. The synthetic peptide, tested in other laboratories (personal communications from H. N. Guttman, D. Malin and L. Oliver, and G. F. Domagk), was found active in the same dose range as in our experiments.

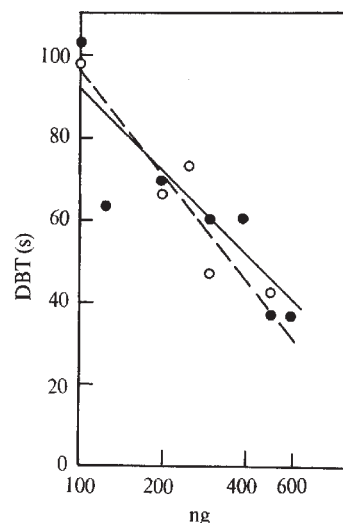


Fig. 4 Dose-response curve of natural (○) and synthetic (●) scotophobin. Abscissa: dose in ng per 20–25 g mouse (log scale); ordinate: seconds in dark box out of total of 180 s (DBT). Before injection DBT was 130–135 s. Solid line, natural scotophobin; broken line, synthetic scotophobin. Regression lines were calculated by the least squares method. Natural scotophobin was tested in groups of twelve mice at each dose; the synthetic peptide was tested in groups of twenty-four mice, except for 150 and 200 ng which were tested in twenty mice each and 400 ng tested in twelve.

The synthetic peptide and its tryptic fragments T_1 and T_2 showed the following R_F values on thin layer chromatography with our standard solvent system: 0.5–0.57, 0.30, 0.37, respectively. The dansyl derivative of the synthetic peptide had R_F 0.16 in both dimensions. It is therefore highly probable that peptide D has the same structure as the substance isolated from the brain of dark-avoiding rats. We gave the name scotophobin (from the Greek *skotos*, dark, and *phobos*, fear) to this substance.

We propose to synthesize other analogues of scotophobin to find the possible critical sites of the molecule, and to design a radioimmunoassay to replace the behavioural test which, like all bioassays, has a low reliability and was found difficult to replicate by some workers (E. L. Bennett, A. Goldstein, personal communications). It has, however, been successfully used in several other laboratories (refs. 15–18 and personal communication from W. F. Herblin and H. N. Guttman).

Table 2 Mass Spectrometric Determination of Overlapping Peptides

<i>m/e</i> found	<i>m/e</i> calculated	Difference (mMU)	Intensity *	Structure †	Elemental composition
314.0815	314.0863	–4.8	48	Ser-Asp-Asn (minus 2H)	$\text{C}_{11}\text{H}_{14}\text{N}_4\text{O}_7$
226.0692	226.0702	–1.0	60	Asn-Asn (minus 2H)	$\text{C}_8\text{H}_{10}\text{N}_4\text{O}_4$
244.0862	244.0933	–7.1	3	Asn-Glu (plus H)	$\text{C}_9\text{H}_{14}\text{N}_3\text{O}_5$
258.1058	258.1090	–4.2	3	Glu-Gln§ (plus H)	$\text{C}_{10}\text{H}_{16}\text{N}_3\text{O}_5$
185.0720	185.0800	–8.0	596	Gln-Gly§	$\text{C}_7\text{H}_{11}\text{N}_3\text{O}_3$
350.0777	350.0939	–16.2	2	Gly-Lys (TFA) ₂ ‡ (plus H minus CO)	$\text{C}_{11}\text{H}_{14}\text{N}_3\text{O}_3\text{F}_6$
310.1163	310.1014	+14.9	<1	Lys (TFA)-Ser ‡ (minus H)	$\text{C}_{11}\text{H}_{15}\text{N}_3\text{O}_4\text{F}_3$
158.0747	158.0691	+5.6	4	Ser-Ala	$\text{C}_6\text{H}_{10}\text{N}_2\text{O}_3$
182.0712	182.0691	+1.9	3	Ala-Gln (minus NH_3)	$\text{C}_8\text{H}_{10}\text{N}_2\text{O}_3$
258.1058	258.1090	–4.2	3	Glu-Gln§ (plus H)	$\text{C}_{10}\text{H}_{16}\text{N}_3\text{O}_5$
185.0720	185.0800	–8.0	596	Gln-Gly§	$\text{C}_7\text{H}_{11}\text{N}_3\text{O}_3$
113.0361	113.0351	+1.0	12	Gly-Gly (minus H)	$\text{C}_4\text{H}_5\text{N}_2\text{O}_2$
235.1034	235.0957	+7.7	13	Gly-Tyr- NH_2 (minus H)	$\text{C}_{11}\text{H}_{13}\text{N}_3\text{O}_3$

* All intensities are normalized to the largest peak (assigned 1,000 arbitrary units).

† In all probability, the dipeptide fragments existed as diketopiperazines.

‡ TFA = trifluoroacetyl. The large difference between calculated and observed masses may be due to the low intensities and to the fact that lysine can fragment in a number of ways. The Lys-Ser linkage, however, is postulated by trypsin hydrolysis and the *N*-terminal determination of its products.

§ These masses were found in both tryptic fragments.

Scotophobin may be the first identified among the many coded molecules into which information has to be converted for being processed by the nervous system¹⁹⁻²². Its real significance cannot be assessed until a number of other behaviour-inducing substances are isolated by us as well as by other laboratories.

This work was supported by the National Institute of Mental Health (G. U.), the National Institute of General Medical Science (D. M. D.) and the Robert A. Welch Foundation (D. M. D. and W. P.). We thank Mrs Faye Johnson for help in the training and testing of animals, Dr I. K. Ho and Mr L. Galvan in the isolation and purification of the material, and Mr G. Holzer in the synthesis.

Received February 8; revised October 18, 1971.

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Comments on the Chemistry of Scotophobin

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Mr Stewart sets out here his comments as referee on the preceding article by Drs Ungar, Desiderio and Parr. These comments represent his remaining reservations after full consultation with the authors.

FIFTEEN months ago Drs Ungar, Desiderio and Parr submitted to *Nature* a short article which announced a remarkable finding. I was asked to referee the article; in correspondence with the authors it became clear that we disagreed on some basic questions. Because of the great import the authors' work will have if valid and because of the widespread attention this work has already received¹⁻¹⁵, it did not seem desirable to delay publication while seeking closer agreement. At the suggestion of the editor of *Nature* therefore and with the cooperation of Drs Ungar, Desiderio and Parr, I present here those of my reservations that remain unresolved.

The authors have generously given me permission to quote from their correspondence concerning their manuscript; these quotations are acknowledged as personal communications.

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How Much Experimental Support is Appropriate?

The authors and I frequently differed over the amount of experimental data required to support their conclusions. On each occasion I favoured giving more data, they favoured less. Because this is a recurring theme of the comments that follow, I summarize my position here.

Many investigators will want to repeat and extend the authors' findings. Their report should therefore contain enough procedural detail to satisfy this need, especially since successful demonstrations of chemical transfer of learning have frequently been hard to repeat. Indeed, in a recent review Dr Ungar concluded that there must be one or more unknown factors which can influence the success of chemical transfer experiments¹⁶. In the face of this uncertainty it seems mandatory to report important experiments in unusually great detail.

In a personal communication, the authors have stated that they intended their present article as a brief letter to *Nature*. I felt that under the circumstances this was not entirely reasonable: their findings have previously been presented in whole or in part in no less than one brief note¹⁷, five abstracts¹⁸⁻²², three symposia²³⁻²⁵ and eight review articles^{16,26-32}. These seventeen articles occupy well over 100 journal pages, but in no case is enough experimental detail given to allow duplication of the important experiments. These preliminary communications have made the work of Dr Ungar and his colleagues

well known to scientists and laymen. Another preliminary communication hardly seems necessary; what is missing is a detailed account.

Biological Assay

The most impressive features of the authors' article are the biological assay and particularly the striking dose-response curves (see their Fig. 4). These are remarkable findings if valid.

I note, however, that no unequivocal relationship between these phenomena and the possibility of a "chemical transfer of learning" is established by the authors' present experiments. The trained donors differed from the controls in more than one way: they have been trained to avoid the dark, and also they had been heavily stressed during the training. Thus any effect induced by the brains of the trained rats could be due either to stress associated with the dark or to stress in general. Only the former could be considered a chemical transfer of learning. An additional control group consisting of stressed but untrained rats would be the minimum required to distinguish between these possibilities. Another point which the authors' experiments do not clarify is the specificity of the effect: the observed decrease in dark-box time could simply be due, for example, to a stimulation of motor activity.

In their present article the authors interpret their results simply as induction of specific behaviour rather than as chemical transfer of learning. Previous reports have not made this distinction^{18,20,25,33}. Regardless of the ultimate explanation, however, the bioassay and the dose-response curves are striking and unexpected effects. If reproducible, they constitute a major discovery.

Can the bioassay be reproduced? This may be difficult to determine since the authors have specified rather few of the parameters of their assay system. In recent reviews^{16,30}, Dr Ungar has listed many factors which he felt could affect the success of a transfer experiment, including the training schedule of the donors, the age of the recipients, the schedule of testing and the interval between administration and testing. It is particularly regrettable that these and similar parameters are not specified in the authors' present article.

The reproducibility of the bioassay does not seem to be settled by the available evidence. On the one hand, Goldstein *et al.* have recently described a thorough but unsuccessful attempt to reproduce the bioassay with the natural material³⁴. They report that they collaborated with Dr Ungar in an unsuccessful attempt to trace the source of the discrepancy between their results and his. Others have also reported negative results^{35,36}. On the other hand, there have been several confirmations of the bioassay using the natural material³⁷⁻⁴¹ and the synthetic material⁴². Some confirmatory studies report effects which are small compared with those observed by Dr Ungar and his co-workers, while others seem to suffer from poor experimental design. Some, however, appear to be sound, and, considered together, these experiments have to be taken quite seriously.

Further research is clearly needed to define conditions sufficient to ensure that the bioassay will be generally repeatable.

Isolation

The procedure used by the authors to isolate active material is shown in their Fig. 1. Repetition of this procedure in other laboratories may prove difficult since certain steps are not completely described in this article or elsewhere. Neither the text nor their Fig. 1 specifies, for example, how much 1.0 M, pH 5.5, ammonium acetate buffer was added to the water phase before addition of alcohol. The composition of the ammonium acetate buffer used to adjust the supernatant to pH 3.7 is not specified, nor is the final ionic strength of the supernatant. The pH of the 10^{-4} M EDTA solution is not

given, nor is the molarity of the pH 6.5 pyridine acetate buffer. Neither the composition nor the pH of the eluant for the 'Sephadex G-25' column is specified.

To make the isolation procedure easier to repeat, I asked the authors to provide, if possible, two additional types of data: the weight and the specific activity of the material at each step of the isolation, and the experimental basis for the assertion that "activity reached its peak at 480 ml.". Dr Ungar stated that he has this information, but that he prefers not to publish it (personal communication). Commenting on his preference he states:

We have records of close to 200 columns done over a period of two years. Since we could not possibly test each of the tubes of the fraction collector separately, we had to pool these in various ways until, by trial and error, we could define the active fraction. A detailed description of this phase of our research would be not only unnecessary but also confusing to those who want to repeat the work.

Some of the 200 experiments referred to by Dr Ungar were probably preliminary, and for these an exact interpretation may not be possible. Preliminary experiments, however, should lead to further experiments for which exact interpretations are possible, and it is just these for which additional data would be useful. It is in any case still unclear how the authors determined where biological activity reached a peak.

Purity

It is important for the authors to demonstrate the chemical purity of the isolated material in order to ensure that their chemical characterization of the material is valid. They report two series of experiments which test the purity of the isolated material and interpret them as showing that "the total amino-acid impurities could not represent more than a fraction of 1%". The first series, however, seems to be quite inconclusive and the second series suggests that in fact the isolated material is contaminated with substantial amounts of impurities.

The first series involves chromatography using ninhydrin to visualize the compounds. The authors observed a single spot in three thin layer systems and in two paper chromatography systems; unfortunately, however, all these experiments were carried out near the threshold of detection. Since they chromatographed between 2.5 and 5 times the minimum detectable amount, and since their postulated structure contains two amino groups, all the experiments are compatible with the presence of one or several impurities each present in amounts up to 0.8 mol/mol of the major component. This first set of experiments, therefore, gives essentially no useful information on the purity of the isolated material.

The authors' second set of experiments, which uses the dansyl group as a fluorescent marker, is more sensitive; contrary to what the authors assert, however, a quantitative analysis of their experiments indicates that the isolated material is impure.

Their conclusion that the isolated material is pure depends on their assumption that the chromatography method they used⁴³ would have detected a single picomole of impurity (0.1 ng of an amino-acid $\approx$ 1 picomole), a point on which they give no experimental evidence. Although the method of Neuhoff *et al.*⁴³ can in certain conditions detect a single picomole, this limit holds only in the most favourable cases and almost certainly does not apply to the authors' experiments. A more realistic limit can be deduced from their own data. In another part of their article they describe the limits of detection for the major component of the isolated material after dansylation as follows: "The absence of this material from untrained brain was further confirmed by the dansyl method which allows its detection at 50 to 100 ng." If the limit of detection for the major component of the isolated material (calculated molecular weight 1,600; 2 dansyl groups) is taken as 20 ng at best, this corresponds to a limit of 20 pmol

of dansyl groups. Thus the additional spots seen at loads of 100 ng or more are evidence for several impurities each present in the amount of 0.4 mol/mol of the major component.

Thus the authors' chromatography experiments do not show that isolated material is pure, but indicate instead that there are several impurities present in the range of 0.4 mol/mol of the major component. The presence of the impurities would have two serious consequences for the interpretation of the rest of their work. (1) It would be arbitrary to attribute the observed biological activity to the major component of the isolated material rather than to one of the impurities. (2) The presence of several components enormously complicates the interpretation of the amino-acid analysis, the degradative chemistry and the mass spectrometry.

Amino-acid Analysis

Discussion of the authors' single amino-acid analysis is complicated by the fact that they do not have access to the original graph from which the results were computed (personal communication). This is a distinct loss since the result they report contains a striking disagreement with theory. They have, in essence, recovered 1.13 g of anhydro amino-acids for every 1.00 g of material hydrolysed (see my Table 1). Unreported amino-acids present in submolar quantities (see below), excess ammonia, salts and absorbed water could only increase this discrepancy. The authors suspect that the error occurred in weighing the 330 µg of isolated material, since they state in a personal communication that "this weight may easily have been 20% off". An estimated weighing error of 20% or some 65 µg is in my opinion exceptionally large, since an ordinary chemical microbalance will weigh with a precision of 1 µg and an accuracy of a few µg. An error in the weighing will cause a corresponding error in every quantitative experiment involving the isolated material, including the dose-response curve. It is true, as the authors point out, that an erroneous weight will not affect the calculated ratios of amino-acids.

Table 1 Weight of Amino-acids found by Drs Ungar, Desiderio and Parr per Microgram of Hydrolysed Material

Amino-acid	Molecular weight	Anhydro molecular weight	nmol found per µg	Weight per µg
Lys	146.19	128.17	0.73	0.094
Asp	133.10	115.08	2.12	0.244
Ser	105.09	87.07	1.33	0.116
Glu	147.13	129.11	2.90	0.374
Gly	75.07	57.05	2.13	0.122
Ala	89.09	71.07	0.65	0.046
Tyr-NH ₂	180.21	162.19	0.81	0.131
Total (in µg/µg of hydrolysed material):				1.127

A second weakness in the amino-acid analysis is the possibility that amino-acids other than those named are present in submolar amounts. The authors state that "no other amino-acids were reported to be present in amounts above 20% of Asp". Since their postulated structure contains 3 residues of aspartic acid, this means only that no other amino-acid occurred in amounts above 60 mol %. Presumably they have no information to contradict the possibility that other amino-acids or peptides are present in amounts up to 0.6 mol/mol of the major component.

Chemical Degradation

Most of the evidence presented by Drs Ungar, Desiderio and Parr for their approximate formula "Ser-(Asp, Glu, Gly)-Lys-Ser-(Ala, Glu, Gly, Tyr)NH₂" comes from degradation

experiments involving enzymatic or chemical hydrolysis followed by dansylation and chromatography. They give so little detail that it is generally impossible to assess the reliability of these crucial experiments. The presence of substantial amounts of impurities in the isolated material, however, poses a very difficult problem for any chromatography experiment. Because there is only a five-fold difference between the limit of detection of the major component and the point at which impurities can be detected, the load applied to the chromatogram is critical. The authors have to steer a course between two hazards: (1) that of applying too little material to the chromatogram and thus overlooking an amino-acid present in molar amounts and (2) that of applying too much material to the chromatogram and erroneously considering an impurity to be a component present in molar amounts. Since the molar ratio of impurity to major component is probably between 1 : 5 and 1 : 2 and since the ratio of least to greatest component in the authors' proposed structure is 1 : 4, there is no obvious way to distinguish between a minor component of the peptide itself and an impurity; the two could well be present at about the same molar concentration. It is probably impossible, therefore, to perform the experiment the authors describe in a way that would allow unambiguous conclusions.

Mass Spectrometric Evidence

The authors and I differ over how many of the original mass spectrometric data ought to be published. Their Table 2 gives only a small fraction, perhaps 1 to 10%, of all the observed peaks. They believe that all the tabulated peaks support their postulated structure, but by publishing only the data consistent with their interpretation and by omitting all the rest, the authors leave little room for an independent evaluation of their reasoning. The practice of reporting only peaks consistent with a particular interpretation is common in mass spectrometry, since most mass spectra contain many uninterpretable peaks. But because the authors' mass spectrum in particular is unusually hard to interpret and because there are so many possible formulae consistent with the chemical evidence (both points are discussed below), the ordinary procedure is not applicable and the mass spectra should be published in full. In spite of repeated urging, the authors have not agreed to this.

For interpretation of their mass spectra the authors rely heavily on the conclusions of their chemical studies. Since almost none of their chemical conclusions is demonstrated in two different ways, a mistake or an ambiguity in any one of the chemical experiments would probably cause an error in the final conclusion. I have argued in a previous section that their chemical evidence is in fact far from conclusive.

If their chemical evidence is accepted at face value, however, an immense number of possibilities remain. Their approximate or partial structure is "Ser-(Asp, Glu, Gly)-Lys-Ser-(Ala, Glu, Gly, Tyr)NH₂", or more exactly, Ser-(Asx₃ Gly_{3-q} Glx_{4-p})-Lys-Ser-(Ala₁ Tyr₁ Gly_q Glx_p)-NH₂, where q=1 or 2 and p=1, 2 or 3. This structure, deduced from the chemical data, includes in all 7,787,520 complete formulae (see my Table 2). The authors use the mass spectrometric data to narrow the field down to the eight possibilities contained in formula 4 of their Fig. 3. But do the data really eliminate all the other 7,787,512 possibilities? Since they report only the data which fit their interpretation, it is impossible to answer this question with certainty. As a practical matter, however, the odds are overwhelming that the answer is no, as the following arguments show.

Mass Spectra: Question of Significance

The authors based the interpretation of their three mass spectra entirely on thirteen selected peaks of low mass ratio, all but two of which were also of low intensity. A typical high-resolution mass spectrum contains, say, 200 such peaks, giving rise

to more than 10^{19} different combinations of thirteen peaks. Furthermore, there are 7,787,520 structural formulae to choose from (see my Table 1), each consistent with all the chemical evidence. What are the odds that on the basis of chance alone one combination of thirteen peaks would agree well with at least one of the structural formulae?

A simple calculation gives the answer. Assume for the sake of convenience that each of the authors' three spectra (one spectrum for the intact peptide and one spectrum for each of the two tryptic fragments) contains 200 peaks of usable intensity between 100 and 350 mass units (MU). Because of the positive mass defect of most elements, the peaks can all be assumed to fall on the positive side of some integral mass number and within 0.2 MU of this number. The authors' root mean square error is ± 0.008 MU (see their Table 2), and so if 600 peaks of the three spectra were distributed randomly in the space of $250 \times 0.2 = 50$ MU, then the peaks with their errors would cover $2 \times 0.008 \times 600 = 9.6$ MU. The probability that these peaks with their experimental errors would cover the mass value corresponding to a particular combination of C, H and N is then about $9.6/50 = 0.192$. The probability of observing at least one formula corresponding to a particular dipeptide (plus or minus 1 or 2 H atoms; 5 possibilities) is $1 - (1 - 0.192)^5 = 0.656$, and the probability of observing 14 dipeptide formulae corresponding to a given pentadecapeptide is $(0.656)^{14} = 0.0027^*$.

Structure of Pseudo-scotophobin

As a test of the general correctness of the above computations, the mass spectrum of an unrelated pentapeptide was examined using the interpretation methods of Drs Ungar, Desiderio and Parr to see whether, under these rules, the spectrum was compatible with one of the 7,787,520 formulae consistent with all of the authors' chemical data. To make the test harder, I restricted myself to structures closely similar to the one given by the authors.

The low-resolution mass spectrum of acetyl-valyl-leucyl-seryl-glutamyl-glycine dimethyl ester is shown in my Fig. 1; it is a typical peptide spectrum in that it contains a rather large number of peaks. From these peaks it proved easy to choose a combination which had the appearance of leading to a unique structural formula. The resulting structure, designated here for convenience "pseudo-scotophobin", is shown in my Table 3 together with the data and the interpretation which led to the structure. This interpretation of the mass spectral data should be compared with the authors' interpretation (their Table 2).

The interpretation for pseudo-scotophobin is superior to the authors' own interpretation in almost every important technical aspect. The average error between computed and observed mass is considerably less for pseudo-scotophobin (root mean square error for pseudo-scotophobin = 3.97 milli-

Table 2 Total Number of Exact Sequences compatible with the Partial Sequence Ser-(Asx₃Gly_{3-q}Glx_{4-p})-Lys-Ser-(Ala₁Tyr₁Gly_qGlx_p) where q=1 or 2 and p=1, 2 or 3

Composition of T ₁	Composition of T ₂	No. of sequences for T ₁ exclusive of amidation	No. of sequences for T ₂ exclusive of amidation	Total No. of sequences including amidation (T ₁ × T ₂ × 2 ⁷)
Ser-(Asx ₃ Gly ₂ Glx ₃)-Lys	Ser-(Ala ₁ Tyr ₁ Gly ₁ Glx ₁)-NH ₂	560	24	1,720,320
Ser-(Asx ₃ Gly ₂ Glx ₂)-Lys	Ser-(Ala ₁ Tyr ₁ Gly ₁ Glx ₂)-NH ₂	210	60	1,612,800
Ser-(Asx ₃ Gly ₂ Glx ₁)-Lys	Ser-(Ala ₁ Tyr ₁ Gly ₁ Glx ₃)-NH ₂	60	120	921,600
Ser-(Asx ₃ Gly ₁ Glx ₃)-Lys	Ser-(Ala ₁ Tyr ₁ Gly ₂ Glx ₁)-NH ₂	140	60	1,075,200
Ser-(Asx ₃ Gly ₁ Glx ₂)-Lys	Ser-(Ala ₁ Tyr ₁ Gly ₂ Glx ₂)-NH ₂	60	180	1,382,400
Ser-(Asx ₃ Gly ₁ Glx ₁)-Lys	Ser-(Ala ₁ Tyr ₁ Gly ₂ Glx ₃)-NH ₂	20	420	1,075,200
				Total: 7,787,520

This calculation leads to a startling conclusion: of randomly chosen pentadecapeptides, approximately three out of every 1,000 would be compatible with the authors' mass spectrometric data. Therefore it could have been predicted that on the basis of chance alone from the 7,787,520 possible formulae many thousands could be found that would be consistent with the mass spectrometric data. That the authors have found such a formula is no surprise, nor is it evidence that the isolated material contains a compound with that structure.

This sort of problem is commonly encountered in interpreting mass spectra, but the authors have greatly aggravated it by basing their interpretation chiefly on small peaks of low mass number, which is contrary to good practice. Also their experimental error is unusually large, which greatly increases the number of possibilities.

A clear implication of these calculations is that there are not merely eight structures, as the authors imply, but rather several thousands which are consistent with both the chemical and the mass spectrometric data. This, if true, would largely vitiate their proof of structure. The prediction can only be tested by reference to the authors' mass spectrometric data, which they have so far been unwilling to release.

* The calculation given above is only approximate; a more exact calculation leads to the same general conclusion (my unpublished computations).

mass units; r.m.s. error for the authors' structure = 7.93 mMU). The greatest error is also less (8.2 mMU compared with 16.2 mMU). The average mass number of the peaks is greater for pseudo-scotophobin than for the authors' isolated material (268 vs. 232), and the average intensity is greater (geometric mean intensity for pseudo-scotophobin = 17.1; geometric mean for the authors' structure = 14.7). The interpretation for pseudo-scotophobin contains no ambiguities, whereas the authors' interpretation contains three (at positions 2, 5 and 11). Finally, there is more overlap in the peptide fragment ions observed for pseudo-scotophobin and therefore supposedly less chance for error.

The point is that in spite of the somewhat more convincing data which can be given in support of the structure of pseudo-scotophobin, the entire interpretation presented in my Table 3 is manifestly false. Indeed it is probable that every structural assignment in my Table 3 is wrong; the only possible exception is the peak assigned to Glu-Gly. This illustrates the point that if one departs from sound practice in interpreting a mass spectrum, it is possible to prove virtually anything.

Mass Spectra: Ambiguities and Errors

Besides the defects discussed above, the authors' interpretation contains some ambiguities and errors.

The demonstrated presence of impurities in the isolated material complicates the interpretation of the mass spectra,

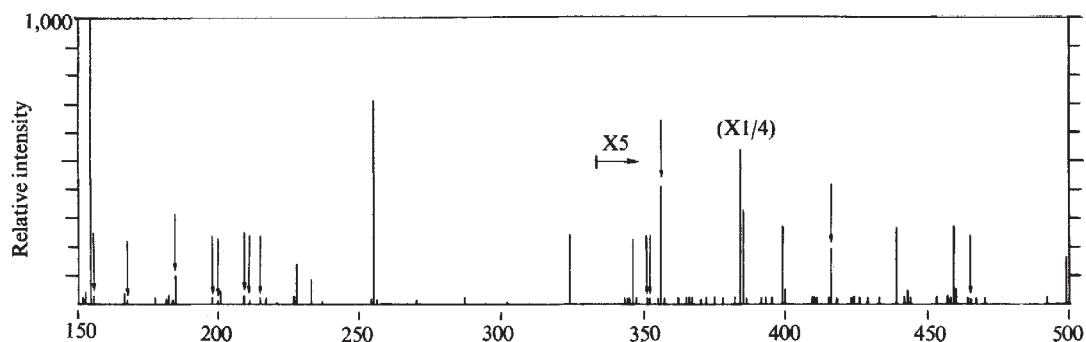


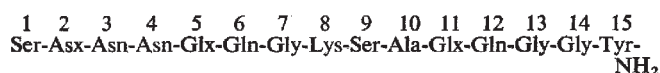
Fig. 1 Line diagram of a low-resolution mass spectrum of acetyl-valyl-leucyl-seryl-glutamyl-glycine dimethyl ester from $m/e=150$ to $m/e=500$. The largest peak in this interval was assigned a relative intensity of 1,000. The arrows mark the peaks whose exact masses were used to deduce the structure of pseudo-scotophobin (Table 3). The low-resolution spectrum was reconstructed from high-resolution data obtained on an AEI MS-902 high-resolution mass spectrometer. The spectrum and the data shown in Table 3 were given by Drs Wipf and McLafferty.

because one cannot be sure that any particular peak belongs to the major component. Some or all the peaks listed in their Table 2 could be due to impurities more volatile than the major component.

Several of the authors' structural interpretations (column 5 of their Table 2) are not unique. Every di- or tripeptide containing asparagine (three of fourteen entries) could equally well be a tri- or tetrapeptide containing glycine. Also, every peptide containing glycine (five of fourteen entries) could in reality be derived by loss of the side chain from a peptide containing any amino-acid other than glycine; loss of the side chain is in fact a common fragmentation pattern. All the intense (>1% of the base peak) peaks observed suffer from at least one of these two ambiguities.

An unnecessary ambiguity is caused by the way in which the authors report the mass spectrometric data. Their Table 2 lists peaks from three different mass spectra but does not show which peaks correspond to which spectra.

Besides these ambiguities their interpretations contain two frank errors. The first is the authors' faulty interpretation of all peaks to which an Asp or a Glu residue is assigned (four of fourteen peaks). The partial structure



deduced from the mass spectra contains ambiguities in positions 2, 5 and 11; the authors feel they cannot say whether the indicated aspartic and glutamic residues in the peptide are present in the amide form or the free acid form. This seems at first to be reasonable: loss of ammonia from an amide residue and loss of methanol from a methyl ester are both common fragmentation patterns and both give rise to the same structure, a substituted ketene. Hence, observation of a ketene is consistent with either the amide or the methyl ester. The error is that the elemental composition of the peaks in question fits neither the substituted ketene, nor the amide, nor the methyl ester. The listed compositions are correct only for the free acid form of the residue, but it is incorrect to assume that the carboxyl group will be free after treatment with diazomethane. The observed peaks are not confirmation of the authors' proposed structure; they could probably be interpreted theoretically on the basis of this structure, but do not constitute evidence in its favour. Incidentally, the peaks which the authors attribute to glutamyl peptides in the free acid form could more easily be explained as aspartyl peptides in the form of the methyl ester.

The second error concerns the peak attributed to Gly-Lys (TFA)₂. It is hard to see how this fragment could be formed from either the peptide or its tryptic fragments. Once again, the peak could probably be interpreted on the basis of their

Table 3 Structure of Pseudo-Scotophobin Deduced from the Spectrum of an Unrelated Peptide

m/e found	m/e calculated	Difference (mMU)	Intensity	Structure	Elemental composition
200.0673	200.0671	+0.2	11	Ser-Asn (minus H)	$C_7H_{10}N_3O_4$
211.0615	211.0593	+2.2	17	Asn-Asx	$C_8H_6N_3O_4$
356.1447	356.1444	+0.3	83	Asn-Asn-Gln	$C_{13}H_{20}N_6O_6$
209.0805	209.0800	+0.5	20	Gln-Glx (minus 2H) (minus CO)	$C_9H_{11}N_3O_3$
198.0657	198.0640	+1.7	20	Glu-Gly (minus 2H)	$C_8H_{10}N_2O_4$
185.1215	185.1164	+5.1	35	Gly-Lys	$C_8H_{15}N_3O_2$
351.1252	351.1280	-2.8	<1	Gly-Lys(TFA)-Ser (minus H_2O) (plus H)	$C_{13}H_{18}N_4O_4F_3$
156.0581	156.0535	+4.6	11	Ser-Ala (minus 2H)	$C_6H_8N_2O_3$
215.0935	215.0906	+2.9	11	Ser-Ala-Gly	$C_8H_{13}N_3O_4$
352.1323	352.1257	+6.6	<1	Gly-Gly-Glx-Gln (minus H)	$C_{14}H_{18}N_5O_6$
168.0453	168.0535	-8.2	17	Gly-Glx	$C_7H_8N_2O_3$
465.2319	465.2335	-1.6	2	Gly-Gln-Gln-Tyr-NH ₂ (minus CO) (plus H)	$C_{20}H_{31}N_7O_6$
416.1525	416.1570	-4.5	40	Glx-Gln-Tyr-NH ₂ (minus 2H)	$C_{19}H_{22}N_5O_6$

Deduced structure = Ser-Asn-Asn-Asn-Gln-Glu-Gly-Lys-Ser-Ala-Gly-Gly-Gln-Gln-Tyr-NH₂.

proposed structure, but could not be considered evidence for this structure.

Characterization of Synthetic Material

The authors' chemical synthesis of the pentadecapeptide Ser-Asp-Asn-Asn-Gln-Gly-Lys-Ser-Ala-Gln-Gln-Gly-Gly-Tyr-NH₂ seems to conform to good chemical standards; the material they isolated probably has the desired structure. The ratios from amino-acid analysis are quite good. Apart from the ratios, no data concerning purity are given, so the synthetic material may be contaminated with small amounts of homologues.

An essential part of a valid proof of structure by synthesis is a careful chemical comparison of the natural with the synthetic material. The value of the comparison carried out by Drs Ungar, Desiderio and Parr is weakened by the probable lack of purity of the isolated material and the possible lack of purity of the synthetic material. Their comparison is in any case too cursory to prove the identity of even the major components of the two materials: they have obtained the R_F s of the synthetic material in only a single one-dimensional and a single two-dimensional system, and they have obtained the R_F s of the tryptic fragments in only one system. They have not performed the crucial experiment of co-chromatographing the natural with the synthetic material (personal communication).

A particularly striking omission is the authors' failure to report high-resolution mass spectrometric data for the synthetic peptide. These data should not be hard to obtain and, if reported in full together with the corresponding data for the natural material, would be a searching test of identity.

Even without using the mass spectrometer it should be easy to obtain strong evidence for identity or dissimilarity of the two materials. One could obtain R_F values for the synthetic material and its tryptic fragments in several solvents, then co-chromatograph the isolated with the synthetic material in these solvents. High-voltage electrophoresis in one dimension and chromatography in the other of both materials and of their tryptic digests would be a good test of identity. The electrophoretic separations in particular would be useful in checking the assignment of residues 2, 5 and 11, which the authors consider ambiguous.

Chemical Differences caused by Training

The authors describe two chromatography experiments to show that the active material is present in brains of trained rats and absent from brains of untrained rats, an important point if valid. The experiments, however, are technically difficult, and the authors do not seem to have taken the precautions that would ensure that their conclusions are correct.

They have failed to provide experimental evidence on two important points. (1) Is the isolation procedure consistently reliable when applied to the brains of trained rats? Specifically, how many times has the present isolation procedure been applied to the brains of trained rats, and what were the yields in each case? Have there been failures? (2) Is the isolation procedure reliable in the absence of a bioassay? Since the authors were necessarily without a bioassay in working with brains of untrained rats, they may have inadvertently discarded the material they wished to isolate during one of the purification steps. One way to answer this question would be through several "blind" attempts to isolate the active material from the brains of both trained and untrained rats without using the bioassay. The authors should report such data if available.

Discrepancies with Previous Publications

Dr Ungar has elsewhere described the limited acceptance which has been given to his and his colleagues' work²⁸. He attributes this to a prior commitment by critics of his work to theories of learning other than those implied by his results⁴⁴. It seems, however, that there must be at least two other

reasons why the work has often been received with scepticism. (1) Almost all the chemistry reported by Dr Ungar and his co-workers has been published in the form of conclusions with neither the data nor the reasoning being given. (2) The chemical conclusions have differed from one publication to the next, and in most cases no explanation for these differences has been given.

Perhaps the most striking discrepancy concerns the authors' previous description of the mass spectra¹⁷, which are described in their present article in more detail. Discussing these mass spectra, Desiderio, Ungar and White previously said¹⁷: "Accurate masses were obtained to within four millimass units. This accuracy permitted the assignment of a unique elemental composition to most fragments".

The implication is clearly that the maximum error in the authors' measured masses was 4 mMU and that their assignments in particular agreed at least this closely with theory. How far this is from the facts as the authors now present them can be judged from their Table 2: eight of the fourteen differences are well outside the limit of 4 mMU previously claimed. The average error is more than one and a half times what was claimed before as the maximum error. Nor is it true that in most cases a unique elemental composition can be deduced. My Table 4 shows that regardless of whether a limit of 4 mMU or the more realistic limit of 10 mMU is adopted, there is not a single case in which a unique elemental composition can be assigned; the average number of possibilities is 4.2 and 10.0 respectively. Note that for several reasons these are conservative estimates: low error limits, neglect of fluorine and exclusion of compounds containing more than 6 nitrogen or oxygen atoms all bias the results towards low estimates. In three out of fourteen cases, in fact, the choices represented by even the larger figure for the number of possibilities do not include the authors' own assignments.

Table 4 Number of C, H, N and O Formulae fitting Mass Spectrometric Data

<i>m/e</i> found	Computed error mMU	No. within ± 4 mMU	No. within ± 10 mMU
314.0815	-4.8	4	9
226.0692	-1.0	5	10
244.0862	-7.1	4	11
258.1058	-4.2	5	13
185.0720	-8.0	4	10
350.0777	-16.2	5	10
310.1163	+14.9	4	11
158.0747	+5.6	2	7
182.0712	+1.9	4	10
258.1058	-4.2	5	13
185.0720	-8.0	4	10
113.0361	+1.0	3	4
235.1034	+7.7	6	12
Average	7.93 (r.m.s.)	4.23	10.0

This table gives the number of formulae containing only C, H, N and O which fall within either ± 4 mMU or ± 10 mMU of the peaks reported by Drs Ungar, Desiderio and Parr. Only formulas which contain no more than six atoms of either N or O and which meet certain other tests of chemical reasonableness⁴⁵ are included.

The final discrepancy in the mass spectrometric data concerns the authors' sample preparation: Drs Desiderio, Ungar and White reported previously¹⁷ that they first esterified and then trifluoroacetylated the peptides, but now they report that they used the reverse sequence. Which statement is correct?

Another striking set of discrepancies concerns the amino-acid composition of the active material, which has been reported differently in different publications. The authors' present amino-acid composition is 1 Ala, 3 Asx, 4 Glx, 3 Gly, 1 Lys, 2 Ser and 1 Tyr. But in ref. 28 Dr Ungar reports that "a quantitative amino-acid analysis done by Dr W. C. Starbuck gave the following composition: 1 Ala, 3 Asx, 4 Glx, 1 Lys, 2 Ser, 1 Tyr". Glycine is absent from this analysis and no

experimental data are given. In ref. 24, however, Drs Ungar, Ho, Galvan and Desiderio report that "a quantitative amino-acid analysis (for which we are indebted to Dr W. C. Starbuck) gave the following composition: 4 Glx, 3 Asx, 3 Gly, 2 Ser, Lys, Tyr". This formulation includes glycine but excludes alanine! Again no data are given. The analysis lacking alanine has been reported in one other publication in identical form²¹ again without experimental data.

The reported amino-acid compositions of the two tryptic peptides have likewise varied remarkably. The composition of the first tryptic peptide T₁ has been once reported as Asx, Glx, Lys, and Ser³⁰, and once as Asx, Glx, Gly, and Lys²⁸, and is now given as Asx, Glx, Gly, Lys and Ser. The composition of T₂ has been twice reported as Glx, Gly Ser, and Tyr^{21,24}, once as Glx, Gly and Tyr²⁸, and now as Ala, Glx, Gly, Ser and Tyr.

It should be stated that the purification of peptides present in trace amounts is not an easy task and that in several cases the presence of residual impurities in the first preparations of biologically active peptides has led to errors. This number of discrepant amino-acid compositions, however, all originating from the same group within 3 years, is probably unprecedented. Furthermore, none of the authors' earlier results can reasonably be attributed to impurities: their earlier analyses without exception showed fewer amino-acids than the current analysis. Purification of a peptide mixture can remove amino-acids from the analysis but not add them. If the discrepancies are to be attributed to impurities, then the authors' most recent preparation must be the most impure.

The reported size of the active material has also varied with the publication. In ref. 33 it is stated that "the transfer factor is probably a basic peptide with between six and ten amino-acid residues", but no data are given. This same estimate is repeated in another publication¹⁸, again without data. In ref. 25, however, Drs Ungar and Fjerdingstad say of the active material that its "elution characteristics suggested the same comparatively low molecular weight" as a material thought to have "a molecular weight of around 1,200". The fragmentary data presented, however, seem quite inconclusive. In ref. 19 the "known chemical properties" of the transfer factor are taken to indicate a molecular weight of "about 1,200". No data are presented.

In ref. 23 Dr Ungar states that "gel-filtration on 'Sephadex G-25' indicates a chain length of 8-12 amino-acid residues". This estimate is repeated in several publications^{16,30} but in no case with data. In ref. 16 Dr Ungar states, without qualification or evidence, that "the substance capable of inducing dark avoidance in mice has been identified as a tetradecapeptide".

In another publication which mentions the 'Sephadex G-25' experiments, the results are interpreted somewhat differently²⁹: "All three of these preparations were fractionated by gel filtration on 'Sephadex G-25' and their elution characteristics were compatible with the assumption that they consist of peptides with 8 to 16 amino-acid residues". Here again, no experimental data are given. Finally, in ref. 21 Drs Ungar, Ho and Galvan state without qualification that "the active substance is a comparatively small molecule (MW 1750)"; no data are given. In their present publication the authors conclude that the substance is a pentadecapeptide (with a calculated molecular weight of 1,600). More disturbing than the range in reported sizes (which covers about a factor of two) is the consistent absence of published experimental support.

The authors' present structure for the active material is

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Ser-Asp-Asn-Asn-Gln-Gln-Gly-Lys-Ser-Ala-Gln-Gln-Gly-Gly-Tyr-NH₂

In ref. 45 the complete structure was reported without qualification as

1 2 3 4 5 6 7 8 9 10 11 12 13 14
Ser-Asp-Asn-Asn-Glu-Gln-Gly-Lys-Ser-Gln-Gly-Gly-Gln-Tyr

Alanine is missing from this formula, the order of amino-acids in the second tryptic peptide is different from that in the present structure, and the tyrosine residue is present in the form of the free acid. No data are given. The complete sequence has been several times reported^{27,28,30} without qualification as

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Ser-Asp-Asn-Asn-Glu-Gln-Gly-Lys-Ser-Ala-Glu-Gln-Gly-Gly-Tyr-NH₂

This of course is the authors' sequence (B), which they now consider unlikely because of an attempted synthesis and because of ambiguities in the mass spectrometric data. In the cited publications, however, these ambiguities are not even mentioned and no experimental support of any sort is presented.

The specific activity of the isolated material has also been variously reported. In their first announcement of the chemical transfer of learned fear of the dark (revised February 16, 1968; published March 30, 1968)³³ Drs Ungar, Galvan and Clark were able to state that "at the present state of purity, one unit of activity corresponds to approximately 5 µg of partially purified extract". (A unit of activity was the amount required to reduce average time in the dark box to 90 s.) But by April 1968 the specific activity had fallen by a factor of six²³: "The transfer factor of dark avoidance, whose purification is the most advanced, has to be administered at the dose of 30 µg per mouse for a discernible effect." No explanation for the discrepancy is given.

Another apparent discrepancy, which, however, has been discussed by Dr Ungar, concerns the behaviour of the compound on partition between phenol and water. The first publications indicated that after dialysis the active factor present in the dialysate partitioned entirely into phenol^{29,33}. Then Dr Ungar reported that if the material was not first dialysed, activity could be found in both phases¹⁶. In fact, for crude material partitioned between phenol and water "both preparations were about equally active".³⁰ Most recently he reports that for undialysed material "we also have done 115 phenol partitions. The activity in the phenol phase varied from 0 to 20%" (personal communication). Dr Ungar has proposed that the apparent difference in the behaviour of dialysed and undialysed material might be due to formation of a complex between the transfer factor and RNA²³: "Some of our recent studies with more concentrated solutions of the transfer factors suggest that they can form complexes with acidic material, as basic peptides are known to do. This suggests that the transfer peptides may combine with ribosomal RNA." Later he presented evidence for this assertion^{16,25}, and in the authors' present publication it is simply taken as fact. But the experimental evidence Dr Ungar has reported seems to me quite inconclusive, and until this point is settled I think the behaviour of the transfer factor on phenol partition under various conditions must be considered unexplained. Although a conclusive test will require use of the isolated material, it would be extremely simple to determine at least whether or not the synthetic material forms a complex with RNA.

This uncertainty about the phenol-water partition properties is important since these properties were used in the isolation procedure. Dr Ungar and his colleagues have isolated the transfer factor in two different ways. A previous publication²³ outlined an isolation procedure very similar to the one shown in the authors' Fig. 1, except that the dialysis was performed before the phenol-water partition. In the earlier procedure the phenol phase was kept, the water phase discarded²³; in the current procedure the phenol is discarded, and the water phase is kept.

Another discrepancy concerning the isolation involves the yields for the steps shown by the authors in their Fig. 1. In ref. 30 an analogous set of yields is given which differs consistently, however, by a factor of 1,000. Which set of figures is correct?

A final discrepancy concerns the crucial question of the reproducibility of the biological assay. In their initial announce-

ment of the phenomenon³³, Drs Ungar, Galvan and Clark stated:

The experiments reported here are easily and rapidly reproducible and yield unequivocal results which clearly demonstrate the possibility of a purely chemical transfer of some types of acquired information.

More recently¹⁶, however, under the heading "Trial and Error for Optimal Conditions", Dr Ungar stated:

Not all our experiments were successful and occasional failures have been experienced by other workers in this area. To succeed, too many conditions, most of them unknown, have to be fulfilled and, if even one of them is neglected, the whole experiment may fail. The probability of a bad experiment is always greater than that of a successful one. It is, therefore, essential to conduct several studies, pool their data and assess the overall significance of the results before drawing any conclusion.

Personal Evaluation

I present below a personal evaluation of the authors' data and some recommendations for future experiments.

The synthesis of the pentadecapeptide is essentially sound. All the work involving the natural material, however, including the isolation itself, the proof of purity, the amino-acid analysis, the chemical and mass spectrometric evidence and the comparison of the natural with the synthetic material are open to serious question. The isolation is not described in enough detail to ensure that other investigators will be able to repeat it successfully. The authors' intended proof of purity indicates that the isolated material is a mixture and suggests that the molar ratio of major to minor component is between 5:1 and 2:1. The amino-acid analysis contains a serious internal contradiction, and the authors are lacking certain crucial information on the possible presence of minor components. The value of the chemical evidence is largely vitiated by the probable presence of impurities. The mass spectrometric evidence, which is crucial for the proof of structure, contains an extraordinary number of errors. The comparison of the isolated with the synthetic material is cursory and inconclusive.

The weaknesses in those parts of the article which deal with the isolated material are so grave, in my opinion, that the authors' conclusions are more likely false than true. The authors have cited the scarceness of the natural material as a factor conditioning the design of some of the experiments I have criticized. I suggest, however, that their entire isolation procedure, even including the training of the rats, is simpler than the corresponding procedures for many other natural products. I think that in any case the authors' conclusions about the chemistry of the isolated material will not be widely accepted until the work is repeated with more documentation and with more attention to important detail.

Repetition of the isolation ought not, however, to be the first priority: the most important questions concern the bioassay. Can the bioassay be successfully repeated outside the authors' laboratory? If so, what are the conditions necessary to minimize its variability? It would be contrary to good sense to embark on a difficult and expensive isolation or to develop a radioimmunoassay if the validity of the bioassay is still in question.

What is therefore probably the most important consequence of the present paper by Drs Ungar, Desiderio and Parr is the possibility of checking the reproducibility of their bioassay in a simple way. Previously it was necessary to train exhaustively a large number of rats and to prepare extracts of their brains; both procedures depend on many variables¹⁶. With the advent of a synthetic material with the same activity as the natural material, it should be comparatively simple to determine whether the assay can be reproduced by others and, if so, to determine which variables influence the result. Because of the obvious importance of this question, it is to be hoped that several groups will be willing to undertake the task of determining the reproducibility of the bioassay.

If the bioassay is reproducible, I think that in spite of the

residual uncertainties about the chemistry the next goal should be to determine by more rigorously controlled experiments whether the observed effect is a true chemical transfer of learning or is a consequence of some less specific mechanism. The former case would be of immense importance. It is just this importance which calls for careful attempts to repeat the authors' work.

Received March 3, 1972.

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Drs Ungar, Desiderio and Parr reply as follows: It would be impossible, within the limits of five days and 1,500 words granted to us, to answer in detail the criticisms for which Mr Stewart was given over a year and apparently unlimited space. We hope, however, to produce enough arguments to reverse his evaluation that "our conclusions are more likely false than true".

Mr Stewart questions repeatedly the validity of the bioassay which is, of course, the cornerstone of the whole work. Although he quotes the six successful replications of our experiments and concedes that "considered together these experiments

have to be taken seriously", he questions the validity of some of them. On the other hand, he takes at their face value the three unsuccessful experiments, as if their negativity made them immune to criticism. He also asks for control experiments, all of which have been done and published (see his references 23, 25, 30 and another article that escaped his attention: (G. Ungar, in *Chemical Transfer of Learned Information* (edit. by E. J. Fjerdingsstad), 31; North-Holland, 1971).

We are ready to plead guilty for omission of some details in the description of our isolation procedures, partly because of what we believed to be space limitations, partly because they were published elsewhere or were not considered critical. We fail to understand, however, why it is "still unclear how the authors determined where biological activity reached a peak". Our article states that, throughout the purification, the fractions were tested by bioassay and Fig. 1 defines the active fraction of the gel filtration effluent and indicates the yield.

Perhaps the most important criticism concerns the purity of the final product. First of all, expressing impurities as molar ratios is completely misleading when applied to peptides and amino-acids. At superficial reading, the molar ratio of "between 5:1 and 2:1" suggests the presence of up to 33% impurities, disregarding the fact that the major component has a molecular weight of 1,600 against about 100 for the probable impurities. Furthermore, when a chromatographic spot remains unresolved after passage through six solvent systems, it is highly improbable that it still contains significant amounts of impurities.

Even if one accepts molar ratios as valid ways of evaluating impurities, our results, obtained by the dansyl method, show about 125 pmol of dansylscotophobin accompanied by five spots representing about 1 pmol each. Three of the spots were identified as amino-acids. Two others were unknown at the time the article was written but since then were found to resist hydrolysis and probably consist in traces of polyamines. Even by the most generous estimate, the impurities cannot represent more than a few per cent. Qualitative analysis by the dansyl method repeated in different batches of material always gave the same amino-acids. For a while we were uncertain about the presence of alanine; its dansyl derivative was sometimes difficult to detect and the quantitative analysis gave low values. This explains some of the "inconsistencies" listed by Mr Stewart (more of which below). After making a big case out of our admittedly inaccurate weighing, we are glad to note his agreement that "an erroneous weight will not affect the calculated ratios of amino-acids".

Contrary to Mr Stewart's belief, the experiments showing chemical differences caused by training are about the most clearcut and easy experiments to do and interpret. They were repeated more than a dozen times with the dansyl method which can detect scotophobin in as little as 100 mg of trained brain (containing about 25 ng) and were consistently negative with even as much as 5 g of untrained brain.

Our interpretation of the mass spectrometric data is closely linked with the chemical data obtained previously. Only in these conditions could we, with the small amount of material at our disposal, reach the conclusions stated in the article. The calculations of Table 2 represent a purely theoretical exercise which ignores the actual experimental procedure. They are also misleading because they do not take into account the presence of amide groups. As soon as asparagine and glutamine were found in the spectra, the number of sequences in T_1 and T_2 was reduced to a manageable few hundred possibilities. This was a much more reasonable situation than the one suggested by the table, the last column of which is, of course, meaningless and deliberately distorted. Once the overlapping peptides were found, the number of possibilities was drastically reduced and ended up with 8. We did not publish the whole mass spectrum because listing more than 1,000 seven-digit numbers seemed to be beyond the permissible space limits.

As for the "pseudo-scotophobin" diversion, it is a good example of what happens when mass spectra are interpreted out of the context of known chemical data and, contrary to "sound practice", the smallest peaks of the spectrum are deliberately chosen.

One must keep in mind that at the time the work was done, in 1969, the methodology required about 1 μ mol of material and could not go beyond the molecular weight of 1,000. We did each spectrum with slightly over 10 nmol, near or above the molecular weight limit.

It is evident that correctness of the sequence had to be confirmed by the identification of the synthetic peptide with the natural material. Mr Stewart is not satisfied with the identical R_f values obtained with the whole peptide and its tryptic fragments. By confusing the proof with what was to be proved, he says that the value of the tests "is weakened by the probable lack of purity of the isolated material and the possible lack of purity of the synthetic material". We have recently performed what Mr Stewart calls "the crucial experiment": we co-chromatographed, after dansylation, synthetic scotophobin with partially purified trained rat brain extract. We obtained a single spot. The most important element of identification, of course, is the biological activity. This was done by us and has now been repeatedly confirmed by others. All this, however, does not give us the certainty that scotophobin is a unique peptide; there may be several scotophobins with slightly different structures, as found for some of the peptide hormones.

It is expected from a referee to assume the role of devil's advocate. This may include systematic overestimation of the negative aspects and underestimation of the positive aspects of a paper but definitely not a misleading interpretation of the results or the misrepresentation of the normal development of a research as "discrepancies" by taking publications out of their chronological sequence. Like everybody else, we had to re-evaluate constantly our interpretations in the light of new data. If our hesitation concerning alanine is taken into account, and if we except the typographical error in ref. 30 (which would have been easy to detect by counting the residues) and the misreading of a sentence in ref. 28, all the compositions are identical. Our estimate of the size of the peptide kept increasing until the number of amino-acids was ascertained. The estimation of specific activity did fluctuate and, when we switched from the initial small-scale experiments to large-scale purification, it dropped temporarily. At that time, we also had to re-evaluate the 100% reliability of the test because we experienced some failures (4.7%). In some of the tentative sequences published before confirmation by synthesis, alanine was left out (for reasons given above) or the original mass spectrometric data were given. The differences in the results of phenol partition depend on whether it is performed before or after dialysis. As for the reproach of not giving exhaustive technical details in the publications quoted by Mr Stewart, it is explained by the nature of these publications. By his own descriptions, these were: "one brief note, five abstracts, three symposia and three review articles", types of publications that do not usually allow space for such details. They were meant to give the then current state of the research and the tentative interpretation it allowed.

We wondered what may have prompted Mr Stewart to go beyond the normal duties of the referee. One clue to this may be in his first fifteen references. No useful purpose can be served by listing more or less distorted accounts of our work in ephemeral publications except to point to the undue attention given to it by the lay press. We cannot apologize for this because we have no control over it; once something is deemed "newsworthy", it is taken over by the mass media and unwillingness to cooperate with them could only result in an increased inaccuracy of the reporting. Whether this explains fully the special treatment given our paper or not, we only hope that it will not discourage other workers from entering new and controversial areas of research.

LETTERS TO NATURE

PHYSICAL SCIENCES

Floating Orbits, Superradiant Scattering and the Black-hole Bomb

Penrose¹ and Christodoulou² have shown how, in principle, rotational energy can be extracted from a black hole by orbiting and fissioning particles. Recently, Misner³ has pointed out that waves can also extract rotational energy ("superradiant scattering" in which an impinging wave is amplified as it scatters off a rotating hole). As one application of superradiant scattering, Misner has suggested the possible existence of "floating orbits", that is, orbits in which a particle radiatively extracts energy from the hole at the same rate as it radiates energy to infinity; thereby it experiences zero net radiation reaction.

Here we point out a second application of superradiant scattering which we call the "black-hole bomb". We also present the chief results of quantitative analyses of superradiant scattering, floating orbits, and the black-hole bomb, for the case of scalar waves. Quantitative calculations are restricted to the scalar case because the scalar wave equation is separable⁴ in the Kerr gravitational field of a black hole, whereas the gravitational wave equation appears not to be. We expect the gravitational case to resemble the scalar case qualitatively if not quantitatively.

The scalar wave equation

$$\square \Phi = 4\pi T \quad (1)$$

(T a scalar charge density) separates in Boyer-Lindquist coordinates (S. A. T., unpublished) by writing

$$\Phi = e^{-i\omega t} e^{im\varphi} S^m_l(\theta) \psi(r)/r \quad (2)$$

with S^m_l an oblate spheroidal harmonic (D. R. Brill and colleagues, unpublished). We define a new radial coordinate r^* by $dr^*/dr = r^2/(r^2 - 2Mr + a^2)$, so that the equation for the radial function takes the form

$$d^2\psi/dr^{*2} - W(r^*)\psi = (\text{source term}) \quad (3)$$

The mass of the black hole is M and its angular momentum is aM . The effective potential $W(r^*)$ is negative at infinity and near the event horizon $r=r_+$, so travelling waves exist in those regions. In between, $W(r^*)$ is positive, that is, it becomes a potential barrier (J. M. Bardeen, W. H. P. and S. A. T., unpublished).

At infinity the asymptotic solutions for ψ are $\exp[-i\omega(t \pm r^*)]$ corresponding to ingoing ("+") and outgoing ("−") waves. By convention we set $G=c=1$; also we take the real part of Φ as the physical field, which permits the convention $\omega \geq 0$ without loss of generality. On the horizon the asymptotic solutions are $\exp[-i(\omega t + k r^*)]$ where $k = [-W(r^* = -\infty)]^{1/2}$. The correct boundary condition on the horizon is not that the waves appear ingoing in the coordinate frame, but rather that the wave be physically ingoing in the frames of all physical observers, who are dragged around the hole by its rotation. If $m > 0$ and $0 < \omega < m\omega_{\text{horizon}}$, where $\omega_{\text{horizon}} \equiv (\text{angular velocity of "dragging" at the horizon}) = (a/2Mr_{\text{horizon}})$, this physically ingoing condition corresponds to a "coordinate outgoing" wave, $\exp[-i(\omega t - k r^*)]$ (C. M. Misner, unpublished).

We now consider a wave which is incident on the black hole. Normally, a part of the wave's energy reflects off the potential barrier $W(r^*)$, while the rest leaks through and is lost down the hole, so that the outgoing wave is weaker than the ingoing wave. If, however, m and ω are in the anomalous range $0 < \omega < m\omega_{\text{horizon}}$, the wave on the inside of the barrier is coordinate outgoing, it reinforces the reflected wave on the outside of the barrier, and there is thus more outgoing wave energy than ingoing. The extra energy comes from the rotational energy of the black hole. The amount of amplification is never very large, because there is always a potential barrier separating the travelling-wave regions.

Fig. 1 shows the results of our numerical integrations of equation (3) for the most favourable case, a maximally rotating black hole with $a=M$. The maximal amplification is a few tenths of a per cent in energy and occurs for low modes [$l=m \sim \mathcal{O}(1)$] and for wave frequencies $\omega \sim (0.8 \text{ to } 1.0) m\omega_{\text{horizon}}$. For a maximally rotating hole of mass M ,

$$\omega_{\text{horizon}} \approx 10^5 \text{ rad/s } (M_{\odot}/M) \quad (4)$$

By itself, a few tenths of a per cent is unimpressive; but any amplification mechanism admits improvement by positive feedback. To illustrate, in a rather speculative vein, we propose the "black-hole bomb" (closely related to a recent suggestion of Zel'dovich⁶): locate a rotating black hole and construct a spherical mirror around it. The mirror must reflect low-frequency radio waves (equation (4); and we now make the transition from scalar to electromagnetic fields) with reflectivity $\geq 99.8\%$, so that in one reflexion and subsequent superradiant scattering there is a net amplification. The system is then

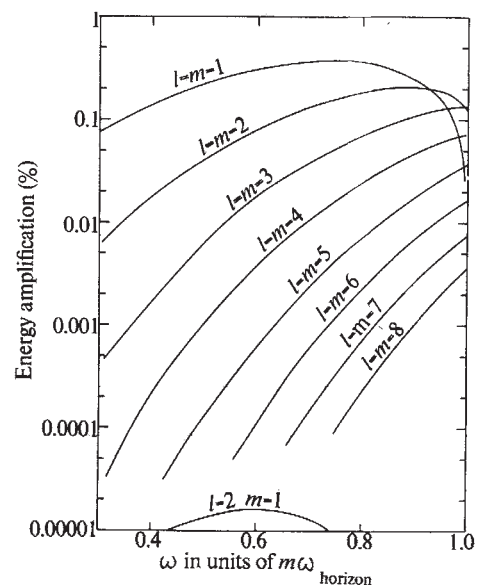


Fig. 1 Superradiant scattering of scalar radiation by maximally-rotating black hole. Radiation modes with axial eigenvalue $m > 0$ and angular frequency $\omega < m\omega_{\text{horizon}}$ are amplified by the hole, not absorbed by it. The fractional wave energy added by the hole is here shown as a function of wave frequency for the most favourable modes.

unstable against a number of exponentially growing electromagnetic modes which will be initiated by random "seed fields" (thermal noise). Because a typical amplification in one reflexion is $\sim 10^{-3}$, the e -folding time is roughly $\tau \sim 10^3 L/c$ where L is the radius of the mirror. For ease of construction, the mirror should not be too close to the hole; for a hole of $M_\odot$ an appropriate choice might be $L \sim 10^3$ km, so that $\tau \sim 3$ s. As the mode grows, electromagnetic pressure on the mirror increases until the mirror explodes, releasing the trapped electromagnetic energy in a time $\sim L/c$. This is the black-hole bomb. Alternatively a port hole in the mirror can be periodically opened, and the resultant radio flux rectified and used as a source of electric power.

Others may care to speculate on the possibility that nature provides her own mirror. The amplified wave frequencies are far below the plasma frequency of the interstellar medium, so that waves would reflect off the boundary of an evacuated cavity surrounding the hole; we are tempted to invoke radiation pressure to maintain the evacuation.

We turn now to "floating orbits". If a particle is in a stable circular orbit around the hole, it generates radiation both outward to infinity as a source term in equation (3), and also (physically) inward into the hole. If the particle is in a direct orbit, co-rotating with the hole, it generates only modes with $m > 0$. For holes with $a > 0.359 M$, radiation from all direct, stable, circular orbits satisfied the anomalous boundary conditions $0 < \omega < m\omega_{\text{horizon}}$ for all l, m . The radiation energy balance of a particle in such an orbit has two parts: the power radiated to infinity, and the power extracted from (not deposited into) the rotating hole. The question of floating depends on the detailed numerical balance of these contributions. If at some radius more energy is extracted than is radiated, the particle will gradually spiral outward until the energy credits and debits are in balance. At this "floating" radius, the particle gradually radiates away the black hole's rotational energy; as a decreases, the radius of the lowest stable orbit moves outward with respect to the floating radius. When the two are equal, floating ceases, and the particle plunges into the hole.

The results of our calculations for scalar radiation are as follows: a system whose dominant radiation is in $m=1$ modes can float around any black hole with $a \gtrsim 0.985 M$; for an $a=m$ hole, the floating radius is at $r \approx 1.4 M$. A system whose dominant modes are $m=2$ can float for $a \gtrsim 0.9995$; for $a=M$ the floating radius is $r \approx 1.16 M$. Systems radiating substantially in $m \geq 3$ modes cannot float at any radius for any $a \leq M$. In the particular case of a point particle in a circular equatorial orbit, it is not difficult to calculate the relative coupling of the source to various modes, and exhibit the energy "balance sheet". Table 1 shows this for the most favourable of all scalar-wave cases: an $a=M$ hole, with the particle very near the lowest stable orbit, $x \equiv r-M \ll M$. Although the first two modes give a net credit, the particle couples too strongly to non-floating modes and there is no net floating. If the particle were smeared out in azimuthal angle ϕ , these higher modes would be suppressed and floating would occur.

For the physical case of gravitational (not scalar) radiation, there is no $l=m=1$ radiation, and the numerical details for higher modes will be different. We do not know if there will be floating for the $l=m=2$ mode (or higher modes for that matter); our scalar results suggest only that gravitational floating is not implausible, and might conceivably enter into the dynamics of material processes near rotating black holes. (Some recent unpublished work by D. M. Chitre and R. H. Price, and by M. Davis and colleagues, suggests that source coupling to high, presumably non-floating, modes is weaker for gravitational than for scalar fields.)

Finally, we mention a curious aspect of our numerical results: in Fig. 1, the amplification factor with $l=m > 1$ does not go to zero as $\omega \rightarrow m\omega_{\text{horizon}}$. Because the amplification factor is negative for $\omega > m\omega_{\text{horizon}}$ it must be a discontinuous function of frequency at $m\omega_{\text{horizon}}$. This discontinuity is an artefact of taking $a=M$. For a slightly lower value, we expect

Table 1 Energy Balance for Scalar Radiation from a Particle in Close Circular Orbit

Mode (l, m)	Power from hole* (credit)	Power lost to infinity* (debit)	Net energy loss (or gain)
1, 1	(0.074)	≈ 0	(0.074)
2, 2	(0.081)	0.032	(0.049)
3, 3	(0.062)	0.070	0.008
4, 4	(0.042)	0.089	0.047
5, 5	(0.027)	0.091	0.064
6, 6	(0.016)	0.087	0.071
7, 7	(0.010)	0.081	0.071
8, 8	(0.006)	0.073	0.067
All modes with $l > m$	≈ 0	≈ 0	≈ 0
Total for modes $l \leq 8$	(0.318)	0.523	0.205

* In units of $(c^5/G) (\mu/M)^2 x$ where $x = (c^2 r/GM - 1) \ll 1$ and μ is the particle's scalar charge. It is an artefact of the scalar case that power $\rightarrow 0$ as $x \rightarrow 0$.

the discontinuity to disappear, with the curves of Fig. 1 showing a sharp turnover very near $\omega/m\omega_{\text{horizon}} = 1$.

We thank C. W. Misner, P. L. Chrzanowski, and K. S. Thorne for discussions. One of us (W. H. P.) thanks the Fannie and John Hertz Foundation for support. This work was supported in part by the US National Science Foundation.

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Received May 15, 1972.

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Whistling Beaches and Seabed Sand Transport

EXPERIMENTS on the whistling sand of Porth Oer, Caernarvonshire, show that the property of whistling or squeaking, when kicked, scuffed or walked on, arises from the very narrow particle size distribution, combined with fairly spherical particle shape¹. These two factors enable the sand to yield in shear along thin slip planes, instead of throughout its bulk, and the drag of uniform particles over one another results in an audible note of regular pitch. There is a tendency for the dilation caused by one particle riding up and over its neighbour to be communicated along the shear plane, so that all the particles at the plane move in unison.

There are several other beaches on which whistling sand occurs. Since carrying out the work reported in ref. 1 we have collected data concerning 32 sites on the coast of the British Isles where such sand may be found. We have visited all of these sites and have taken shape and size distribution measurements on sand samples. These will be published in full later.

We wish now to draw attention to a close correlation between the occurrence of whistling sand and the position of the landward end of "bed-load partings" in the sand of the continental shelf². Kenyon and Stride² report sand movements on the seabed which are caused by both tidal (oscillatory) transport and current (unidirectional) transport. They followed such movements in detail by analysing a large number of wide-scan echo-sounding records of the seabed obtained by the oceanographical survey ships RRS Discovery I and RRS Discovery II on coastal waters survey voyages between 1958 and 1969. The records show sand waves, sand ribbons and sand patches, from which the sand transport directions may be obtained. Certain lines are, in effect, "watersheds" for the flow of sand over the seabed, sand flowing away on each side roughly at

90° to the watershed line. These lines are called bed-load partings. There are also bed-load convergences—lines towards which sand moves from opposite sides. Kenyon and Stride point out that the sand transport paths are not necessarily followed in detail by all or indeed any sand grains; grains enter or leave the paths at any point, and may remain stationary, as a deposit, if they reach an equilibrium position. Precise limits cannot be placed on position or length of the bed-load partings, and they are subject to local temporary changes caused by storm currents.

The bed-load partings and the locations at which whistling sand occurs are shown in Fig. 1. Whistling sand occurs at the landward ends of the bed partings: *A*, near the Orkneys; *B*, between Islay and Antrim; *C*, between Caernarvonshire and Wicklow; *D*, in the Bristol Channel; *E*, between Bournemouth and the Channel Islands; and *G*, off the Northumberland coast. This gives a good correlation between the landward ends of the bed-load partings and whistling beaches.

Parting *F*, adjacent to the coast of Norfolk, is the only one given by Kenyon and Stride which did not appear to have a whistling sand location near its landward end. We therefore examined beaches on the Norfolk coast to confirm the proposed correlation. The sand at Sea Palling proved to whistle quite well; it lies almost exactly on the parting line. The beaches at Happisburgh and others northward as far as Hunstanton were silent, with the possible exception of Holme, as were those at Winterton and southward to Great Yarmouth.

In spite of this evidence in favour of the correlation, several exceptions and discrepancies remain. On the east coast of Scotland, near Montrose and at Cruden Bay, there is whistling sand but no bed-load parting. There is, however, a bed-load

convergence east of Peterhead. On the west coast of Scotland, whistling sand occurs at Clashnessie and at Gruinard Bay, both in Wester Ross; at Morar and Loch Ailort in Inverness; and—a famous example, first noted by Miller³—at Camas Sgiotaig on the Isle of Eigg. But the Minch and the inshore waters near the Small Isles were not surveyed by the wide-scan method, and, because of the strong tides in the area, it seems probable that such a survey would reveal small partings. By analogy with the major partings which arise because of the sea movement around the whole of the British Isles, one might expect subsidiary partings around islands.

The normal way to make sand whistle in the laboratory is by hand-scuffing the surface of the bulk specimen in a polythene bag. This is not a very reproducible method of applying shear, but with a little practice it becomes quite easy to classify sands as good, medium and poor whistlers. The sands from Keel, Broadsea, Tynninghame and Cullercoats are poor whistlers, and they are rather distant from their bed-load partings. Brittas Bay and Porth Oer sands, on the other hand, are outstandingly good, exceeded only by Eigg. Their particle size distributions were very similar, both peaking at 60 mesh (251 μ m) and both having 48% by weight in the peak fraction, using sieves with a $\sqrt{2}$ progression. They also have very similar shape distributions. Further, their most angular fractions both have true particle densities greater than 2.65 g cm⁻³, the density of quartz. The value for 52 to 60 mesh (295 to 251 μ m) Brittas Bay sand is 2.727 g cm⁻³, and that for the same size fraction of Porth Oer sand is 2.730 g cm⁻³. We removed the calcium carbonate (aragonite, density 2.93 g cm⁻³), which is the cause of the high density, by dissolving it in hydrochloric acid. 38.6% by weight of the Brittas Bay sand and 35.8% of the Porth Oer sand dissolved. It is significant that there are so many points of resemblance between two sands at opposite ends of the well defined bed-load parting line *C* running across the Irish Sea. The sands at opposite ends of bed-load parting *B*, at White Park Bay in Antrim and Port Ellen in Islay, are also very like one another. The densities of the most angular fractions in the 60 to 72 mesh (251 to 210 μ m) range are 2.758 and 2.782 g cm⁻³, respectively, and the corresponding acid soluble fractions are 78.1% and 73.8%. The angular, shelly portions of sand are mentioned here only because they provide an indication that sands at opposite ends of a bed-load parting tend to be similar. The bulk of all the sand collected is almost pure silica (as quartz), with roughly spherical grain shapes.

It seems that sea conditions along bed-load parting lines are favourable to the rounding of sand grains, perhaps because they are locations where sand residence times are very long, although the situation in which the grains are located is not a static one from a fluid mechanics standpoint. Indeed, the fluid movement in these equilibrium regions seems to be such that the size grading of the sand is very precise. Provided that a suitable site is available for the sand to reach a stable beach, a whistling location will occur at the landward end of the sand reservoir on the seabed. It would be interesting to examine seabed sand samples, taken at parting lines in deep water, for size, shape and acoustic properties, because there appears to be a causal link between the parting lines, a very accurate size grading of the sand along them, and whistling sand beaches.

Note added in proof. Since this letter was written, we have visited beaches in Caithness and found whistling sand at the north-east end of the beach at Dunnet Bay, at the opposite end of bed-load parting *A* from Rackwick, Hoy.

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Received January 11, 1972.

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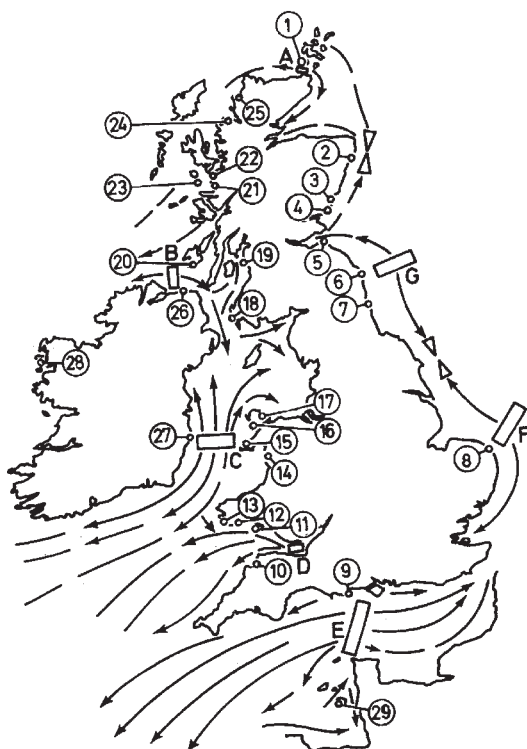


Fig. 1 Rectangles *A* to *G* represent bed-load partings according to Kenyon and Stride². Arrows give the direction of transport of sand along the seabed. Pairs of triangles represent bed-load convergences. Numbers 1 to 29 are whistling sand locations. 1, Rackwick, Hoy; 2, Cruden Bay; 3, Montrose; 4, Lunan Bay; 5, Tynninghame; 6, Bamburgh; 7, Cullercoats; 8, Sea Palling; 9, Studland Bay; 10, Croyde Bay; 11, Rhosili; 12, Penally; 13, Freshwater West; 14, Barmouth; 15, Porth Oer; 16, Newborough; 17, Traeth yr Ora, Anglesey; 18, Broadsea Bay; 19, Ardnish; 20, Port Ellen, Islay; 21, Ardnish (also Roshven); 22, Morar; 23, Camas Sgiotaig, Eigg; 24, Gruinard Bay; 25, Clashnessie; 26, White Park Bay; 27, Brittas Bay (also Silver Strand); 28, Keel; 29, St Brelade's Bay (also Portelet Bay), Jersey.

The Anisotropy of Precipitation Media

A FALLING raindrop may be considered to be an oblate spheroid with its minor axis vertical. A region of precipitation containing many such particles is in effect an array of particles of preferred orientation and will therefore be anisotropic. We have set out to determine the consequences of this effect on radar observations of rain and snow at a radar wavelength of 1.82 cm. Observations of hail at a wavelength of 10.4 cm may also show this effect.

We used a 1.82 cm radar unit at Ottawa, Ontario^{1,2}, and a 10.4 cm radar unit at Penhold, Alberta³. The Ottawa unit has twelve range gates with 0.5 km spacing which makes possible a direct and instantaneous measurement of propagation effects; the Alberta unit has a single movable range gate. Both are capable of transmitting an arbitrarily chosen polarization and of receiving simultaneously the transmitted polarization and that orthogonal to it. The ratio of the powers received in orthogonal channels is known to be closely related to the mean shape and effective dielectric constant of the scattering particles⁴⁻¹⁵.

The in-phase and quadrature components of the correlation between the two receiver signals are also measured. When circular polarization is used these data provide information on the randomness of the particle orientations, and if a preferred orientation exists its direction about the line of sight can be determined.

For example, if there is a single scattering particle with its axis at an angle α anticlockwise from the vertical, the axis lying along OX in an X, Y, Z coordinate system (OZ is along line of sight), then the radar return depends on the scattering matrix components S_{xx} and S_{yy} , and when circular polarization is transmitted the signals in receivers 1 and 2 are given by¹⁶

$$R_1 = R_{10} K_{\pm} TG(\theta, \phi) \zeta_{\pm} \quad (1)$$

$$R_2 = R_{20} K_{\pm} TG(\theta, \phi) \quad (2)$$

Receiver 1 is connected conventionally in the transmitter channel through a duplexer, and responds to the transmitted polarization. A directional coupler network provides an alternate port for receiver 2 which responds to the orthogonal polarization. In equations (1) and (2) $\pm$ indicates RH or LH circular polarizations, R_{10} and R_{20} are reference signals from a vertically polarized transmitter used as a calibration source, $G(\theta, \phi)$ is the antenna directive gain at (θ, ϕ) , and

$$T = \frac{\lambda}{4\pi R^2} \sqrt{\frac{\sigma}{4\pi}} e^{-(\gamma' + \gamma'')R} \quad (3)$$

where λ = wavelength, R = distance to the scatterer, γ' and γ'' are propagation constants for the principal plane polarizations, and the scattering cross section, σ , is $\pi |S_{xx} + S_{yy}|^2$. If $\gamma' = \gamma''$, $\zeta_{\pm} = ve^{i(\delta \pm 2\alpha)}$ where $ve^{i\delta} = (S_{xx} - S_{yy}) / (S_{xx} + S_{yy})$.

In general it can be shown that if $|\zeta_{\pm}|^2 \ll 1$

$$\zeta_{\pm} = ve^{i(\delta \pm 2\alpha)} + 2pe^{i(\chi \pm 2\tau)} \quad (4)$$

where

$$pe^{i\chi} = \tanh \left[\frac{\gamma' - \gamma''}{2} R \right] \quad (5)$$

and τ is the tilt angle of the anisotropy axis from the vertical.

For an assembly of scatterers we define

$$\begin{aligned} W_1 &= \frac{\langle r_1 r_1^* \rangle}{|R_{10}|^2} \\ W_2 &= \frac{\langle r_2 r_2^* \rangle}{|R_{20}|^2} \\ W &= \frac{\langle r_1 r_2^* \rangle}{|R_{10} R_{20}|} \end{aligned} \quad (6)$$

where

$$r_1 = \sum_{i=1}^N R_{1i} \quad r_2 = \sum_{i=1}^N R_{2i}$$

N is the number of particles in the observation cell and the brackets $\langle \rangle$ indicate a time average of what is assumed to be a stationary random process. Then

$$\begin{aligned} \frac{W}{W_2} &= \frac{\langle \sum T_i^2 G^2 (ve^{i(\delta \pm 2\alpha)} + 2pe^{i(\chi \pm 2\tau)}) \rangle}{\langle \sum T_i^2 G^2 \rangle} \\ &= \rho (ve^{i(\delta \pm 2\alpha)} + 2pe^{i(\chi \pm 2\tau)}) \end{aligned} \quad (7)$$

where ρ is the fraction of scatterers with preferred orientation. We assume a model justified for Rayleigh scatterers in which the fractions $(1 - \rho)$ have random orientation and make a null contribution to the term containing $e^{\pm 2i\alpha}$ while the fractions ρ have mean parameters $\bar{v}$, $\bar{\delta}$, and $\bar{\alpha}$. Also

$$\frac{W_1}{W_2} = \frac{|ve^{i(\delta \pm 2\alpha)} + 2pe^{i(\chi \pm 2\tau)}|^2}{2} \quad (8)$$

W_1 , W_2 and W are similar to the elements of the coherency matrix¹⁷. They can be identified with some of the circular polarization parameters introduced by Kušcer and Ribarič¹⁸ and by Sekera¹⁹. W_1 and W_2 are proportional to the mean powers in the same sense and opposite sense circular polarization components while $|W|/\sqrt{W_1 W_2}$ is the correlation coefficient

between the two circular polarization components. W/W_2 is of particular interest because, as is clear from (7), it provides a separation of the scattering and propagation terms. The latter, $2pe^{i(\chi \pm 2\tau)}$, derives from anisotropy along the path between the radar and the observation cell. Because it is known that $\tau \approx 0$, W/W_2 can be related directly to the range dependent parameters ΔA and $A\phi$, which are respectively the differential attenuation and propagation differential phase

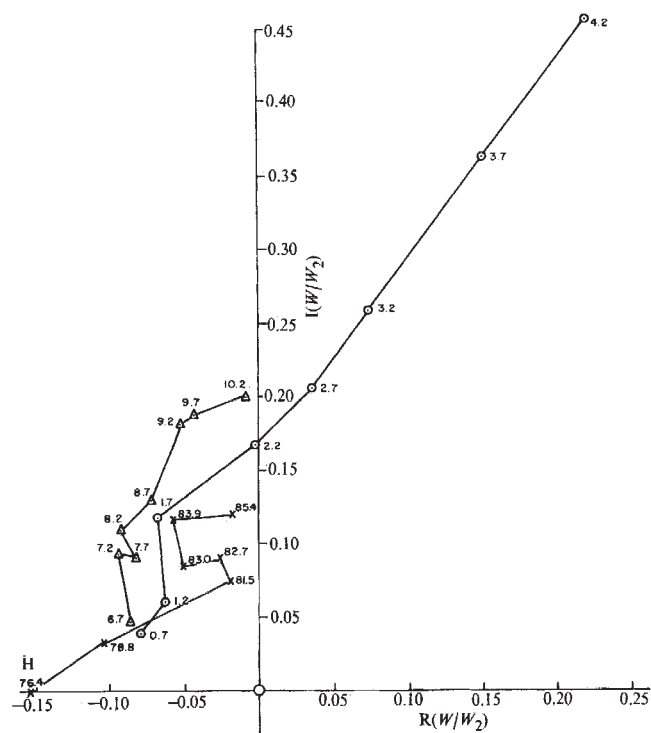


Fig. 1 W/W_2 for Ottawa rainstorms (right hand circular polarization, observation time, 0.4 s), and for an Alberta hailstorm (left hand circular polarization, observation time 1 min). Numbers indicate range in km. Δ , Rainstorm ~ 6 mm/h, Ottawa, July 4, 1970; $\circ$, rainstorm ~ 30 mm/h, Ottawa, July 20, 1970; $\times$, hailstorm, Penhold, June 27, 1970.

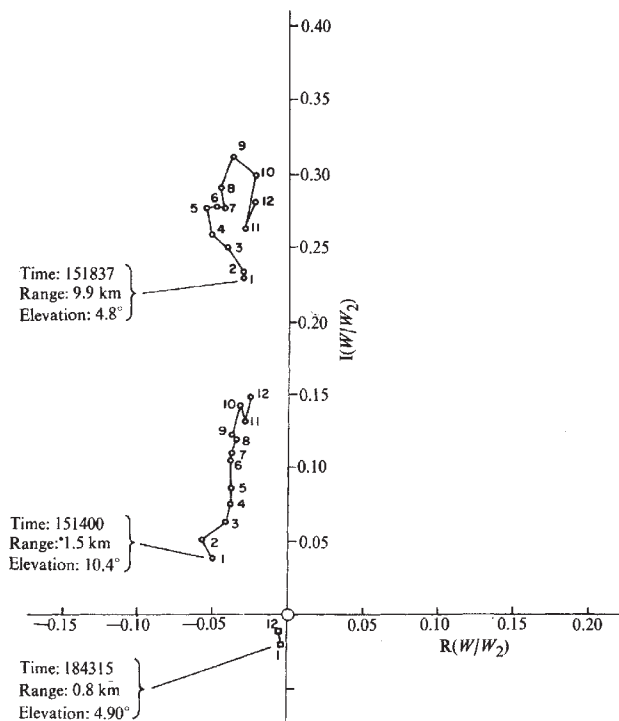


Fig. 2 W/W_2 for Ottawa snowstorms (left hand circular polarization). Numbers indicate successive range gates with spacing of 0.5 km. $\circ$, Heavy snow, December 22, 1970; $\square$, heavy snow, February 13, 1971.

shift in decibels and degrees. If $\rho \bar{v} e^{i(\bar{\alpha} \pm 2\tau)}$ is constant, then between radar range gates M and N ,

$$\frac{\log_e 10}{40} \Delta A + i \frac{\pi}{360} \Delta \phi = \frac{1}{2} \left[\left(\frac{W}{W_2} \right)_N - \left(\frac{W}{W_2} \right)_M \right] \quad (9)$$

that is, $R(W/W_2)$ is associated with ΔA , and $I(W/W_2)$ with $\Delta \phi$.

A comparison of (7) and (8) in situations where p is negligible provides a measure of $\frac{\bar{v}}{\sqrt{v^2}} \rho$, and hence an estimate of ρ , the

fraction of oriented scatterers. All our observations of rain and, some of snow, gave non-zero values, typically 0.75 for rain at 1.8 cm, establishing the existence of anisotropy. The corresponding value at 10.4 cm is typically 0.3.

There has been some previous speculation concerning a preferred orientation of precipitation particles^{7,8,13}. One

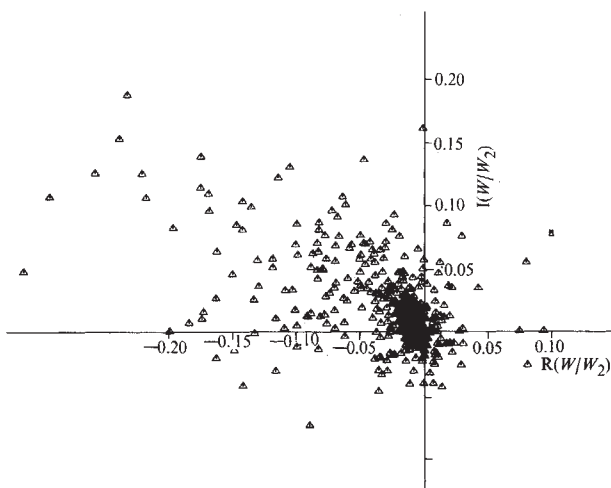


Fig. 3 W/W_2 for an Alberta hailstorm (left hand circular polarization). Penhold, July 28, 1970, No. of points=456.

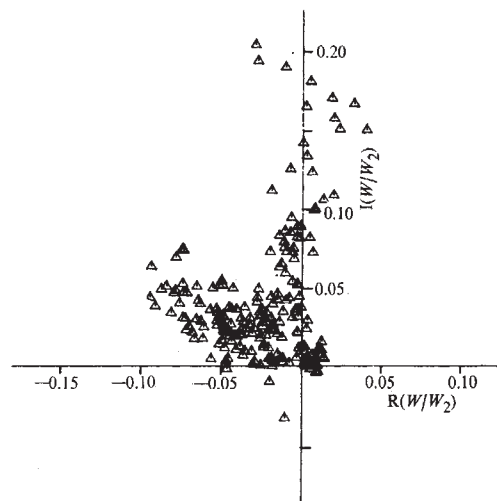


Fig. 4 W/W_2 for an Alberta hailstorm (left hand circular polarization). Penhold, September 6, 1968, No. of points=227.

investigation has been directed toward the detection and measurement of such an effect¹⁰. Oguchi's theoretical analysis²⁰⁻²² has related the preferred orientation to a differential attenuation in the precipitation medium.

Anisotropy implies a range effect whereby with increasing range the term $2pe^{i(\alpha \pm 2\tau)}$ becomes more important and finally dominates in equation (7). Fig. 1 shows this effect for moderate and heavy rain. Estimates of differential attenuation and differential phase shift may be made from Fig. 1 using equation (9); they yield 0.3 dB km⁻¹ and 2.5° km⁻¹ for moderate rain, and 1.0 dB km⁻¹ and 7.5° km⁻¹ for heavy rain. Fig. 2 shows a similar plot for two heavy snowstorms. There was little propagation effect for the storm of February 13, 1971, but a marked anisotropy for the storm of December 22, 1970. The latter showed a negligible differential attenuation but a differential phase shift of 1.1° km⁻¹ for a total of 15° differential phase shift.

Figs. 3 and 4 show plots of W/W_2 for 10.4 cm data. Distributions of points from storms producing rain only lie near the negative real axis. Other observations show a broadened distribution as in Fig. 3. Evidence of two distinct populations is shown in Fig. 4. We suggest tentatively* that the effects shown in Figs. 3 and 4 are produced by a hail medium which is anisotropic at 10.4 cm in a manner similar to rain at 1.8 cm. In fact the Alberta observation of June 27, 1970, shows a range effect (Fig. 1), which corresponds to a differential attenuation and differential phase shift of 0.07 dB km⁻¹ and 0.8° km⁻¹. Ground observations of hail having a flattened shape were made beneath the observation point marked *H*.

In addition to its intrinsic interest the anisotropy effects discussed here have some practical implications: first, communications systems for which the use of orthogonal polarizations is contemplated could encounter serious deterioration when paths cross precipitation areas, although this effect is mitigated by the apparent stability of the tilt angle τ about 0°; second, the optimum polarization for suppression of radar precipitation clutter is elliptical rather than circular—for the storm of December 22, 1970, a measured improvement in clutter suppression as high as 10 dB was obtained by adjusting the transmitted polarization from circular to an axial ratio of 0.76.

*Note added in proof. Recent information suggests that the propagation effects at 10.4 cm may be due to rain accompanying the hail.

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Received October 7, 1971; final revision March 27, 1972.

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Observation of a Frequency Shift during the Amplification of a Narrow Spectrum Light Signal

THE amplification of light signals by a material in an inverted state depends on the gain profile of the amplifying medium and the spectrum of the incoming signal. The fundamental light amplification processes suggest that if a Gaussian (or Lorentzian) shaped narrow spectrum signal is amplified by a wide linewidth amplifier whose line centre does not coincide with that of the signal, frequency shifts occur during the amplification process. In this letter we report a direct experimental observation of a frequency shift occurring during the amplification of a narrow spectrum signal by an amplifier

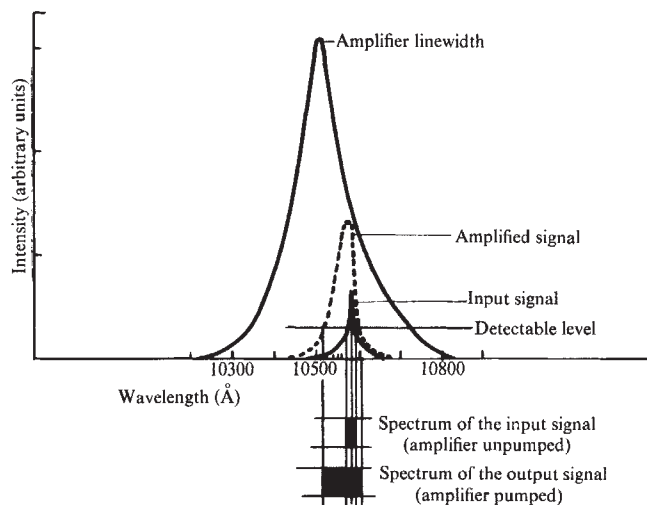


Fig. 2 Schematic diagram demonstrating the amplification results of Fig. 3.

of wide linewidth. The ability of trivalent neodymium to serve as the active ion in different host materials provided us with the necessary experimental conditions because the spectra of the different systems do not coincide exactly.

The experimental arrangement is shown in Fig. 1. The input signals consist of mode-locked pulse trains from an Nd-glass LGN 55 oscillator. These pulses have a time duration of approximately 5 ps. The energies of the input signals used ranged from 30 to 80 mJ, and the amplifying medium was an $\text{Nd}^{3+}:\text{POCl}_3:\text{ZrCl}_4$ liquid system contained in a cell 155 mm long and 7.5 mm in diameter. It was pumped with a double flashlamp arrangement with 'Pyrex' tubes around each flashlamp to eliminate UV radiation. The linewidth of this system is 200 Å at half maximum¹ (Fig. 2), with the centre of the line at 10,520 Å.

The spectra of the signals were taken with a Monospek 1000 grating scanning spectrometer with a Mullard 6929-1 (S1) image converter tube placed in the plane of the exit slit. In order to avoid damaging the replica grating with the high powers of the picosecond pulses, only part of the output beam was reflected into the spectrometer. The exit of the amplifier was imaged at unit magnification onto the input slit of the spectrometer using a 35 cm focal length lens. We used a calibrating lamp to check the accuracy of the recording system,

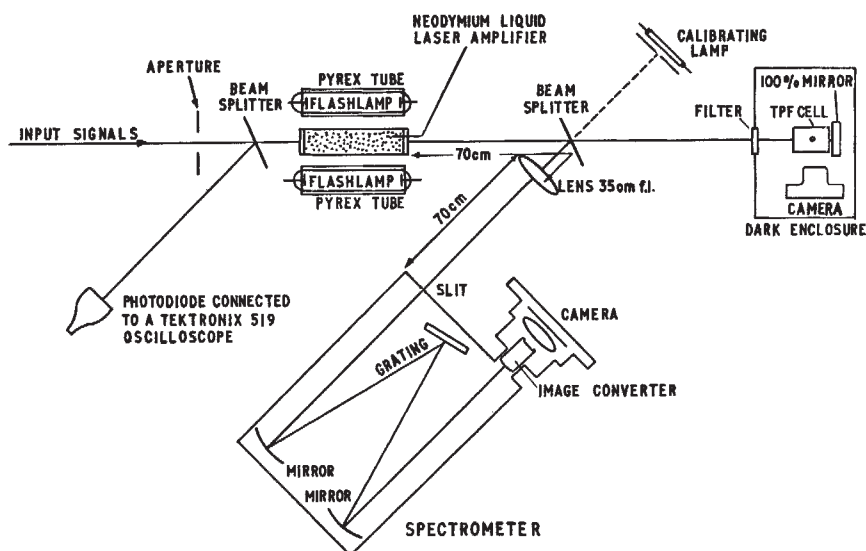


Fig. 1 Experimental arrangement for recording the spectra of the amplified signals.

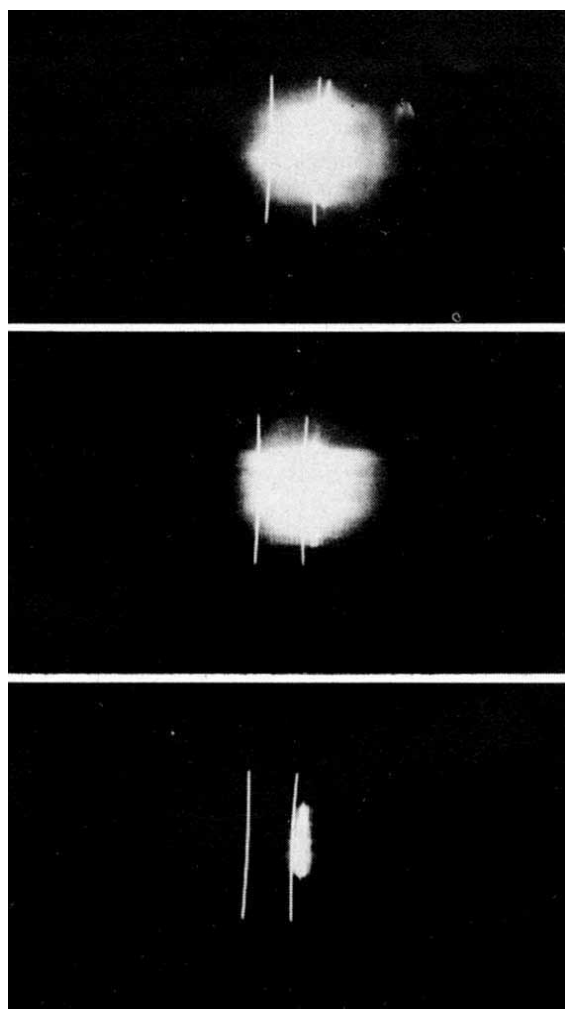


Fig. 3 Lower picture: typical spectrum taken with amplifier unpumped. The lines shown are 10,532 Å (left) and 10,570 Å (right). Top two pictures: typical spectra when the amplifier is fired with energy 722 J. The two lines correspond to the same wavelengths as in the lower picture.

and found that the spectrometer-image converter arrangement is accurate to ± 0.5 Å.

The experimental results are shown in Fig. 3. The two lines correspond to 10,532 Å (left) and 10,570 Å (right). The input signals were first recorded without firing the amplifier, and spectra ranging from 5 to 20 Å wide were obtained, each centred at 10,580 Å. The spectra of the mode-locked pulses from an Nd-glass laser were shown to be largely Gaussian² with half width ~ 5 Å.

When the amplifier is fired the resulting spectrum is asymmetrical about the centre of the input signals. It is shifted towards the centre of the amplifier linewidth and the pictures (Fig. 3) show that the asymmetry is approximately 27 Å. The gain of the amplifier at the time of transmission of the pulses was approximately 6. It is important that the shift never reaches the centre of the amplifier linewidth; this limits the gain obtained from the liquid. In experiments performed with the liquid as an amplifier (refs. 1, 3 and our unpublished work) an Nd-glass oscillator was used. The results described here suggest that the potentialities of the liquid as an amplifier are much better than implied by those experiments.

A schematic demonstration of the amplification process is shown in Fig. 2. The predicted spectra, similar to those obtained in our experiment, are shown below the graphs and are defined by the vertical lines.

Proper calibration of the film to record the intensity distri-

butions of the spectral shifts, and measurements of the gain and energy of the input pulses, will allow important parameters of the cross-relaxation mechanisms in the liquid laser to be calculated by this method. Attempts to evaluate the effects of the spectral mismatch on the gain of the amplifier are now being made.

We thank Dr A. C. Selden for discussions and Mr. S. Ward for technical assistance. The experimental work was performed at the Culham Laboratory, UKAEA.

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BIOLOGICAL SCIENCES

Survival of Common Terrestrial Microorganisms under Simulated Jovian Conditions

WE have investigated the possibility that common terrestrial microorganisms carried by spacecraft can survive entry into the atmosphere of Jupiter. In our experiments we used *Escherichia coli* B, *Serratia marcescens*, *Aerobacter aerogenes*, and *Bacillus subtilis*, routinely cultured in basic mineral medium¹ with glucose (30 g/l.) as the carbon source, or in trypticase soy broth (TSB, 30 g/l.), sterilized by autoclaving at 15 pounds inch⁻² for 15 min. Plating was done using TSB with 1.5% agar.

One-day cultures of the organisms were exposed to simulated Jovian conditions by injecting 1 ml. of each into 5 ml. glass tubes, which were then inserted into 75 ml. stainless steel bombs, fitted with a plug and a needle valve, and flushed free of air by a current of simulated Jovian atmosphere (described later) for 30 s prior to sealing and raising the pressure to the required value. Pressurization was carried out within 1 min of sealing and within 5 min of filling the glass tubes. Immediately after pressurizing the tubes they were transferred to the appropriate temperature environment. A duplicate bomb containing air at atmospheric pressure and at the same temperature as the pressurized bomb was set up as a control. A further control was maintained in air at 20° C to give a measure of the amount of death attributable to standing for 24 h. Immediately prior to filling these tubes, the cultures were sampled and plated out onto agar-TSB plates at dilutions of 10^{-1} – 10^{-8} , 0.1 ml. of each dilution being plated and spread over the surface by sterile glass beads. After incubation at 30° C for 24 h, initial viable counts were obtained from these plates.

After 24 h, the cultures were thawed and depressurized. Depressurization required up to 1 h, as rapid outgassing of the tube contents led to spillage. Immediately the process was complete, dilutions of 10^{-1} – 10^{-8} in TSB or mineral medium were prepared from the cultures and controls. After 24 h incubation at 30° C, viable counts were obtained from the inoculated plates.

As a check on contamination levels a series of 105 plates was inoculated with sterile distilled water previously passed

through the whole procedure of gassing, dilution, etc., and incubated for 7 days in the same incubator as the gassed cultures. Contamination consisted of a total of 16 colonies on 4 plates, appearing on the uppermost plates of the stack at between 6 and 7 days incubation. None of these contaminants produced colonies similar to our test organisms.

In one experiment, the colonies that grew up on plates after being gassed and inoculated as described above were identified by a chemical multitest method ('Enterotube') of known reliability. The organisms thus identified were the same as those used in the experiment.

Table 1 Death of Microorganisms under Simulated Jovian Conditions

Organism	Pressure (atmosphere)	Temperature (°C)	20° C control	Frozen control	Gassed/frozen	Deaths %§
<i>E. coli</i> *	0.1	-196	120 ‡	120	120	0
	2.5	-80	180	80	110	35
	13	-50	130	10	51	67
	50	-13	78	36	2.4	97
	68	0	120	70	90	25
	102	20	78	—¶	20	19
	123	30	180	120	0	100
<i>E. coli</i> †	13	-50	9	11	6.5	28
	50	-13	10	0.007	0.03	99.7
	102	20	28	31	0	100
<i>S. marc.</i> *	0.1	-196	104	102	108	0
	2.5	-80	200	210	110	42
	13	-50	100	110	100	41
	50	-13	87	42	5.4	93
	68	0	100	114	60	50
	102	20	58	—	0	100
	123	30	96	40	0	100
<i>S. marc.</i> †	102	20	24	9.2	10	58
<i>A. aerog.</i> †	13	-50	10	3.0	6	40
	50	-13	7	1.2	2.6	63
	102	20	21	16	0.2	99
<i>B. sub.</i> †	13	-50	2.1	0.75	1.5	28
	50	-13	2.1	0.11	0	100
	102	20	1.9	1.4	0.9	53

* In TSB medium.

† In mineral medium.

‡ All cell counts are viable counts multiplied by 10^{-8} /ml.

§ % death is defined by: (control (20°)—frozen/gassed) $\times$ 100/control (20° C) as viable counts.

¶ Identical to 20° control.

To simulate the Jovian atmosphere we used a mixture of H_2 (56%), He (43%), CH_4 (0.5%), and NH_3 (0.5%). Cultures were gassed prior to freezing in order to permit solution of ammonia; gassing of a frozen culture would not have brought ammonia and cells into contact. The temperature-pressure profile within the Jovian atmosphere is shown in Fig. 1 (see ref. 2), where the points on the curve indicate the conditions in our experiments. Table 1 shows these conditions together with viable counts and the degree of killing of our organisms after a 24 h exposure.

There was significant survival of all organisms at all temperatures, excepting *S. marcescens* and *E. coli* at 20° and 30° C. The killing effect clearly increases at higher temperatures and pressures for all four species, possibly due to the fact that above 0° C ammonia is in equilibrium between liquid cultures and the gaseous phase and hence is readily accessible to the cells. This is not true of frozen cultures. Generally, lower survival in mineral medium may reflect lower initial cell counts. In some cases, gassing seemed to reduce the effect of freezing in both media, although this result requires confirmation. Some variability in the results may be due to traces of

oxygen remaining in the cultures after flushing and gassing. This is difficult to remove without risking some alteration in the cultures, since prolonged flushing with an inert gas would be required.

The survival of these microorganisms under simulated Jovian conditions indicates that there is a very real possibility of the contamination of Jupiter by a non-sterile spacecraft, provided that the planet can also permit growth of such contaminants. We have no data on growth under these conditions, although the apparent ease with which some very common organisms can survive for 24 h suggests that, with

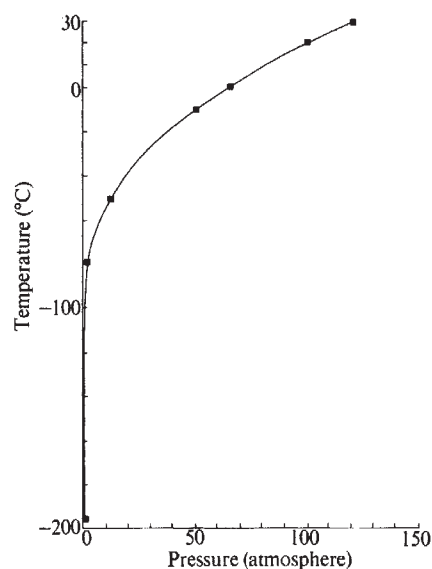


Fig. 1 Assumed temperature-pressure variation within the Jovian atmosphere. Points marked on the curve refer to experimental conditions.

adaptation, growth of anaerobic organisms may be a probability. The synthesis of organic chemicals by electric discharges under simulated Jovian conditions has been demonstrated and the presence of a layer of liquid water clouds suggested², but the only mineral source in the Jovian atmosphere would be from meteoritic precipitation, so the mineral supply for an organism would be very limited. However, the enormous gravitational field of Jupiter and its proximity to the asteroid belt would offset this. Hence there is a very real possibility that terrestrial organisms could reproduce under Jovian conditions. We strongly recommend the sterilization of spacecraft on missions to Jupiter.

We thank Miss JoAnn Williams for technical assistance. This work was supported by a NASA grant.

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Serum and Salivary Antibodies to Glucosyltransferase in Dental Caries in Man

RECENT evidence strongly suggests that dental caries is an infective disease caused predominantly by *Streptococcus mutans*¹⁻³. This organism has two major characteristics responsible for its cariogenicity: first, in the presence of fermentable carbohydrates it is capable of rapidly producing acid to below the pH required for dissolving enamel, and second, the organism produces glucosyltransferase which is a constitutive enzyme responsible for the synthesis of extracellular polysaccharides⁴. These polysaccharides are glucans which are commonly referred to in the literature as dextran; they form a major component of the bacterial plaque matrix and may be responsible for adhesion of the bacterial plaque to tooth enamel⁵.

We have investigated the immune response in man to a variety of antigens derived from *S. mutans*, to find a rational basis for immunization against caries. Estimation of immunoglobulins showed a significantly lower level of salivary IgA ($P < 0.01$) and a slightly higher level of serum IgA concentration in subjects with a high caries incidence as compared with those having a low caries experience⁶. Differential serum antibodies to cariogenic streptococci have been found in subjects with different caries experience using cell wall preparations and ultrasonicates of the bacteria^{7,8}. A correlation between serum antibodies to cell walls of cariogenic streptococci and the index of decayed, missing and filled teeth (DMF) has also been reported⁹.

In view of the importance of glucans in adhesion of dental bacterial plaque to tooth surfaces and their synthesis by glucosyltransferase, we explored the possibility that antibodies may be formed to this enzyme. Immunity to dental caries might be related to specific serum or salivary antibodies which inhibit the enzyme function. If this hypothesis were correct it would then be possible to immunize with a suitable enzyme preparation to elicit active immunity against the enzyme and this would prove to be an effective measure in preventing dental caries.

The usual method of indicating whether antibodies play a role in a disease is to establish a differential antibody level between patients and controls. In dental caries this approach is not applicable as virtually the entire population suffers or has suffered from caries, so that the level of antibodies must be related to the extent of the disease. A series of seventy-two subjects was divided into two groups: (a) a "passive" group consisting of thirty-seven subjects who were either caries free or had filled teeth not in need of any conservative treatment, and (b) an "active" group, each of whom had at least one carious lesion in addition to any treated caries. This division was intended to distinguish between the effects of past treated caries and present untreated caries on the antibody response.

Samples of serum and parotid saliva were collected from each subject and stored at -20°C for antibody assay. The enzyme was prepared from the supernatant of a culture of *S. mutans* and purified by hydroxylapatite chromatography¹⁰. Antibodies in both serum and parotid saliva were assayed by the passive haemagglutination technique. The starting dilution for serum was 1:10 and for saliva 1:2.

Serum antibodies were detected in all subjects, with titres ranging from 1:10 to 1:640. The passive group revealed a significant negative correlation between antibody titre and the DMF index ($P < 0.01$) so that as DMF increased the titre decreased, whereas a positive correlation was observed in the active group (Table 1). Different relationships were found with saliva; antibodies were present in titres of up to 1:64 and these showed a significant positive correlation in the passive group ($P < 0.05$). The salivary titres in the active group did not correlate with the DMF index and in only five of the

Table 1 Coefficients of Correlation between Antibody Titre to Glucosyltransferase and DMF Index

	Group	No. of subjects	Correlation coefficient	Significance <i>P</i>
Serum	Passive	37	-0.493	<0.01
	Active	35	+0.404	<0.02
Parotid saliva	Passive	32	+0.350	<0.05
	Active	31	-0.201	N.S.

thirty-one subjects was the titre greater than 1:4, as compared with fifteen of thirty-four in the passive group ($P < 0.02$).

The serum antibody titres in the passive group could be interpreted on the basis of a protective function in that the highest levels are found in those who have experienced least disease. A further interpretation of this relationship is that exposure to glucosyltransferase early in life leads to immunization and that, in those who respond with high antibody levels, little or no caries develops. In others the level developed may not be high enough to be protective as suggested by the subjects with a high DMF in this group. In the active group subjects with a DMF > 10 have a significantly raised titre, in comparison with those in the passive group ($P < 0.01$). It is possible that persons with a high incidence of caries have an immunological defect in that they are unable to sustain raised levels of antibody, which fall after caries is removed and the subject may again become susceptible to the disease. Detailed interpretation, however, must await the results of long term sequential immunological studies now in progress.

It has been suggested that repeated immunization is needed to achieve high levels of antibodies in secretions^{11,12}. Indeed, the positive correlation between salivary antibodies and the DMF in the passive group may reflect the cumulative caries experience, so that the titre is greatest in those who have had repeated carious attacks, and presumably repeated immunization with the enzyme. The low titres in the active group may indicate either that caries may appear when the salivary antibody level falls below a critical level, or that the titre becomes depressed following onset of caries.

In the oral cavity immunoglobulins from saliva are in direct contact with teeth, and immunoglobulins from serum are found in the crevicular fluid which surrounds the necks of the teeth¹³, so both sources of antibodies could participate in caries immunity. If antibodies to glucosyltransferase were to inhibit the enzyme activity then immunization with glucosyltransferase might prove a valuable measure in preventing dental caries.

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Restricted Host Range of a Feline Leukaemia Virus

FELINE leukaemia viruses (FeLV) from cats with naturally occurring haematopoietic tumours grow in feline cells in culture¹. In addition, of five viral isolates studied by us, four grew in human cells and one did not^{2,3}. This paper reports experiments on the determinants of the host range of FeLV.

Two isolates were used in this study. The first, FeLV-1, had failed to replicate in human cells while the other, FeLV-5, did. Both viruses were obtained from cats with alimentary lymphosarcoma; in the isolation procedure feline embryonic cells of the FEA line⁴ were inoculated with a tumour homogenate and were subcultured at intervals of 3–4 days for 3 weeks, at which time the culture fluids containing the virus were harvested and were stored at -70°C for subsequent use as inocula. The medium used throughout these experiments was Eagle's MEM with 10% foetal bovine serum (EFB).

Seven separate cultures of human embryonic lung (HEL) cells were used; six were derived from primary cultures and one was the HEL 2,000 line (Flow Laboratories, Ltd).

Infection of HEL and FEA cells by FeLV-1 and FeLV-5 was studied. One ml. of virus was added to 7×10^5 cells in 4 ml. of EFB and the mixture plated in a 5 cm Petri dish. The cells were subcultured every 3–4 days and on the twenty-first day were prepared for electron microscopic examination by methods described earlier¹.

Large numbers of leukaemia virus particles were observed in FEA cells inoculated with FeLV-1 or FeLV-5, and in all seven of the HEL cell lines which were infected with FeLV-5; no virus was found in any of the HEL cells inoculated with FeLV-1.

The quantity of each virus produced in FEA and primary HEL cells was estimated by growing the cells in the presence of ^3H -uridine and determining the amount of radioactivity incorporated into viral particles, as described in the legend to Table 1. Although approximately equal amounts of virus were produced in FEA cells infected with FeLV-1 or FeLV-5, and in human cells infected with FeLV-5, no virus was demonstrable in cells inoculated with FeLV-1. The figure in Table 1 for the latter culture is 4% of the others, which is the level obtained in uninfected cells.

We examined the possibility that the envelope of FeLV-1 is a determinant of its restricted host range. Pseudotypes of the Moloney strain of murine sarcoma virus (MSV) with FeLV-1 or FeLV-5 envelopes, MSV (FeLV-1) and MSV (FeLV-5), were manufactured by the method of Fischinger and O'Connor⁵. By virtue of their FeLV envelopes, these viruses can infect and morphologically transform feline cells so that viral replication is easily recognized. When cultures of feline cells are inoculated with high dilutions of MSV (FeLV), focal areas of transformation are observed. In appropriate conditions the number of foci is proportional to the dilution of virus so that viral infectivity can be assayed, the titre being expressed in focus forming units (FFU). Viral stocks contain both pseudotype sarcoma virus and homologous FeLV, and infection of a cell with both viruses is required for cellular transformation and for the production of progeny viruses.

Both MSV (FeLV-1) and MSV (FeLV-5) transform FEA cells. When HEL cells were inoculated with each virus, however, only MSV (FeLV-5) induced transformation; even when 5×10^5 HEL cells were exposed to as much as 8×10^3 FFU of MSV (FeLV-1) together with 10^5 infectious units of FeLV-1 (the latter as an additional, exogenous helper virus to allow full expression of the sarcoma viruses), no morphological change was seen. This large difference in the susceptibility of HEL and FEA cells to MSV (FeLV-1) was confirmed in another experiment in which FEA and HEL cells were exposed to serial dilutions of MSV (FeLV-1) or MSV (FeLV-5) as described in the legend to Table 2. The medium was changed every 2 days, and on the sixth day the viral infectivity on each cell type was determined. The cells were then harvested and reseeded in known amounts into plates with fresh FEA cells to determine

Table 1 Infection of Feline and Human Cells with FeLV-1 and FeLV-5

Virus type	Cell type*	^3H -c.p.m. at density 1.13–1.18 g cm $^{-3}$ †	C.p.m. per 10^3 cells‡	Particles in e.m.
FeLV-1	FEA	2,049	310	+
FeLV-1	HEL	81	11	—
FeLV-5	FEA	2,186	437	+
FeLV-5	HEL	3,128	626	+
—	FEA	31	6	—
—	HEL	51	9	—

* The cells were infected 21 days previously with FeLV.

† Exponentially growing cells in a 5 cm plate were cultured for 21 h in medium containing $10 \mu\text{Ci/ml}$ of ^3H -uridine (The Radiochemical Centre, Amersham; uridine-5-T; specific activity 29 Ci/mmol). The culture fluid was then removed and spun at 2,000 r.p.m. for 10 min. Three ml. of the supernatant fluid was introduced into a tube containing a 5 ml. 20–50% sucrose gradient and a 5 ml. layer of 10% sucrose. After centrifugation at 36,000 r.p.m. for 90 min in a Beckman SW 40 rotor, the gradient was fractionated and the density and acid-insoluble radioactivity of each fraction was determined; the counts per min in the density region 1.13–1.18 g cm $^{-3}$, in which FeLV is found, are shown.

‡ The cells in each plate were removed with trypsin-EDTA and were counted in a haemocytometer.

Table 2 Efficiency of Plating of MSV (FeLV) on Feline and Human Cells*

Virus type	Cell type	Viral titre (FFU/ml.)	Infective centre assay			
			No. of foci on original plate	No. of cells plated	Mean No. of foci observed	% of cells registered as foci
MSV (FeLV-1)	FEA	5.3×10^3	66	1.6×10^2	32	20
MSV (FeLV-1)	HEL	0	0†	1.0×10^5	0	0
MSV (FeLV-5)	FEA	1.7×10^4	212	1.6×10^2	95	59
MSV (FeLV-5)	HEL	2.0×10^3	25	2.0×10^4	24	0.12

* A mixture of 1.0 ml. of a dilution of MSV (FeLV) of either type, approximately 10^5 infectious units of homologous FeLV in 1.0 ml., and 4.0 ml. of EFB containing 7×10^5 FEA cells were plated in 5 cm plastic Petri dishes. The cells were incubated at 37°C for 2 days, when the medium was replaced with fresh EFB. On the fourth day the medium was changed again and on the sixth day the number of foci in each plate was determined by viewing the cultures in an inverted microscope.

One plate in each series was chosen: the cells were harvested with trypsin-EDTA, were washed 3 times in EFB and were then mixed in appropriate numbers with 7×10^5 FEA cells; the mixture was seeded into each of two 5 cm plates. The medium was changed on the third day and on day 6 the cells were fixed and stained with 1% crystal violet in 10% formalin and the foci in each plate were counted.

† The cells on this plate had been exposed to 5.3×10^3 FFU of MSV (FeLV-1) and the appropriate helper virus.

the proportion of the original cells which were infected with each type of virus and to allow replication of virus which was possibly present in very small amounts in the original cultures.

The results (Table 2) showed that the titre of MSV (FeLV-1), assayed in FEA cells, was 5.3×10^3 FFU/ml. and that no foci were seen in the infected HEL cells. When the latter were harvested and co-cultivated with fresh FEA cells for a further 6 days there was still no evidence of viral growth. The human cells also appeared to be less susceptible to infection with MSV (FeLV-5) although only by a factor of 10 compared with one of more than 5,000 for MSV (FeLV-1).

The results obtained with these sarcoma viruses indicate that it is probably the viral envelope which mediates the ability of FeLV to infect human cells; this might be expected from previous knowledge of the control of the host range of avian RNA tumour viruses of different subgroups by determinants on their viral envelopes^{6,7}.

It has been shown that feline leukaemia viruses can be classified in three subgroups (A, B or C) on the basis of cross-interference tests⁸. Our preliminary results indicate that FeLV-1 is of the A subgroup while FeLV-5 contains viruses of

subgroups A and B; of these, the subgroup A virus cannot grow in human cells. In FeLV-5, and hence in MSV (FeLV-5), subgroup B virus comprises a small proportion of the total: this may explain the low efficiency of plating of MSV (FeLV-5) on HEL cells compared with FEA cells.

The investigation of further isolates of FeLV will confirm whether viruses of one particular subgroup are unable to grow in human cells. If this were so, inoculation of human cells would provide a useful method of differentiating types of FeLV. In addition, in view of the suggested potential hazard of FeLV for laboratory workers, such viral types might be more acceptable as models of feline RNA tumour viruses in laboratories where stringent personnel protection is not possible.

We thank M. F. Brennan and M. Golder for their assistance. This work was supported by the Cancer Research Campaign and the Lady Tata Memorial Trust.

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Received April 4, 1972.

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Loss of DDT from Storage in Human Body Fat

BECAUSE DDT is highly soluble in lipids, and therefore stored preferentially in body fat, it is often said to "accumulate in fat". This phrase incorrectly implies that the storage process is progressive and irreversible. Population surveys have shown no increase in DDT concentration in human fat from US residents over the past 15 yr of continuing contamination of food¹⁻⁴. Feeding experiments have also demonstrated in several mammalian species, including man, that the storage process is reversible⁵⁻⁹. Identification of the metabolite DDA (*bis*[*p*-chlorophenylacetic acid]) in human urine demonstrated the existence of a mechanism for DDT excretion as early as 1946 (ref. 10).

Hayes properly summarized existing knowledge of the excreatability of DDT as "intermediate between that of most drugs and that of lead and other bone seekers"¹¹. He also indicated that the rate of loss of fat-stored chlorinated hydrocarbons increases strikingly with the magnitude of store. Excretion measured at low storage levels will therefore underestimate the capacity of organisms to defend against large intakes of these chemicals.

The ability of man to eliminate fat-stored DDT can, in fact, be described quantitatively. During the past 3 yr, we have monitored adipose storage of DDT in three human subjects who took total oral doses of 3.66 g, 1.83 g and 0.26 g technical DDT at rates of 20 mg per day (for 183 days), 10 mg per day (for 183 days), and 5 mg per day (for 52 days), respectively^{9,12}. These dosages were, of course, superimposed on continuing low level dietary intakes, which we would estimate at 16 µg per day of the DDT isomers combined⁴.

As the technical material consisted of 77% *pp'*DDT and

23% *op'*DDT, total doses of the isomers in the three subjects were:

Subject	<i>pp'</i> DDT g	<i>op'</i> DDT g
1	2.82	0.84
2	1.41	0.42
3	0.20	0.06

Fat biopsy samples were extracted and subjected to column cleanup^{13,14}, then concentrations of *pp'*DDT, *op'*DDT and *pp'*DDE were measured by gas liquid chromatography, using 6-foot 1.5% OV-17/2% QF-1 columns (at 200° C) and electron capture detection⁹. Concentrations were expressed in terms of the hexane extractable lipid of the samples.

Fig. 1 shows the time courses of disappearance of *pp'*DDT from body fat over 1.7 to 2.5 yr from the termination of dosing. The analogous data of Hayes⁸, also derived from subjects who had ingested technical DDT, are included. Because Hayes's measurements were made by a method that did not distinguish between DDT isomers, and his subjects (unlike ours) were in a steady state of DDT storage at the time dosage was terminated, some discrepancies in characteristics of the curves can be expected.

First order constants calculated from the *pp'*DDT decay curves do decline with the level of the initial store. To construct a single decay curve spanning the range of storage levels attained in our three subjects, rates of DDT loss at the mean storage values for the individual curves have been calculated from the first order decay constants. Using these three pairs of values for storage and storage-loss, the following parabolic equation has been fitted:

$$\dot{C} = 0.190 C + 0.0054 C^2 \quad (1)$$

where C = instantaneous rate of decline in lipid *pp'*DDT concentration, and C = instantaneous concentration of *pp'*DDT in lipid. Integration of equation (1) by the Riccati equation yields an expression for the decline of adipose lipid *pp'*DDT over the range of storage levels studied:

$$C = \frac{1}{0.0388 \exp(0.190t) - 0.0284} \quad (2)$$

This composite curve is shown in Fig. 2, along with the data points from which the three segments were derived. This use of the three personal curves to construct a composite curve clearly assumes that differences in individual metabolic capabilities are of lesser significance than the effect of DDT storage level.

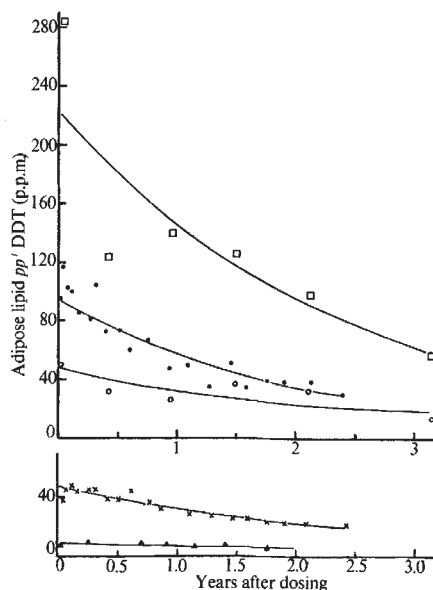


Fig. 1 Disappearance of *pp'*DDT from adipose lipid in subjects previously dosed with technical DDT. □, ○, Six subjects after Hayes⁸; ●, subject 1; ×, subject 2; ▲, subject 3.

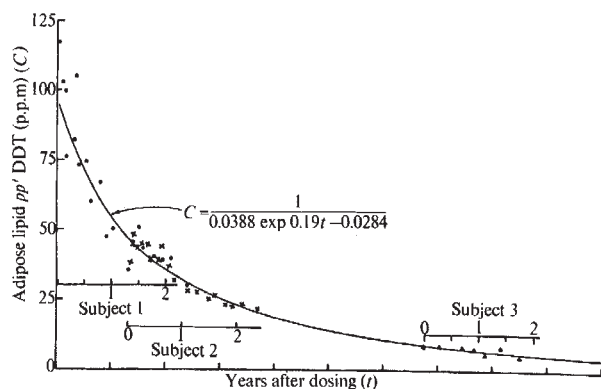


Fig. 2 Composite time course of loss of *pp'* DDT from adipose lipid in three subjects.

The curves for Hayes's subjects at high and low storage levels can be treated similarly, yielding a curve that descends somewhat less steeply in the high range and a little faster at low storage levels than our own curve:

$$C = \frac{1}{0.01045 \exp (0.243t) - 0.00597} \quad (3)$$

An approximate composite curve for storage loss of *pp'* DDT in our subjects is given in Fig. 3. It indicates much faster loss than was found in the case of the *pp'* isomer. Because the later data points approach pre-dose levels of *op'* DDT in the fat, the flat later portion of the curve may reflect continuing dietary intake of *op'* DDT. This feature contrasts with our curves for the *pp'* isomer, all of which lie well above pre-dose values after 2.5 yr, and are probably influenced little by continuing dietary intake of *pp'* DDT.

Is the DDT disappearing from body fat excreted, or is it converted to the even more storable DDE?⁹

The time courses of adipose lipid *pp'* DDE (Fig. 4) in Hayes's subjects and our own (including a fourth for whom we have limited data) indicate a definite upward trend, but the proportion of DDT ultimately converted to DDE is small. Thus, 3 yr after the end of dosing, Hayes's highest dosed subjects had shown an increase of 44 p.p.m. DDE, while DDT had decreased 244 p.p.m. As initial storage level declines, absolute conversion, and the percentage of DDT converted to DDE, decline. At the lowest storage levels, very little trend in adipose *pp'* DDE is thus far evident.

Excretion of *pp'* DDA in the urine certainly continues following termination of DDT dosing¹². The total amount of urinary DDA excreted has not, however, accounted for more than one-third (and usually much less) of the DDT lost in our

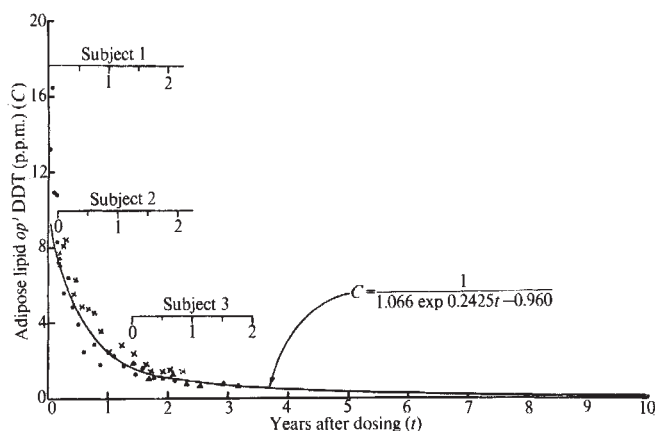


Fig. 3 Composite time course of loss of *op'* DDT from adipose lipid in three subjects.

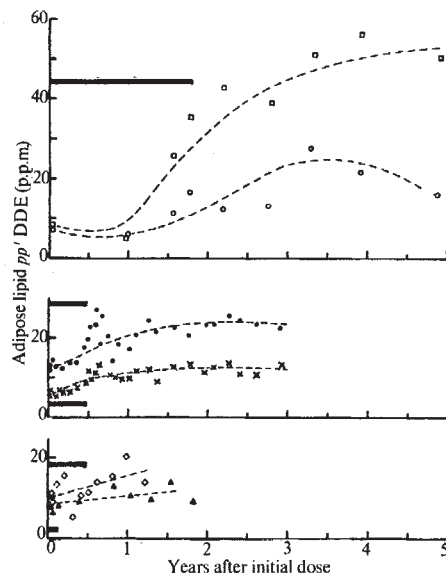


Fig. 4 Adipose lipid *pp'* DDE during and after oral dosing with technical DDT. —, Dosing period; □, six subjects, 35 mg per day; ○, six subjects, 3.5 mg per day (after Hayes⁸); ●, subject 1, 20 mg/day; ×, subject 2, 10 mg/day; ▲, subject 3, 5 mg/day; ◇, subject 4, 5 mg/day.

subjects or in Hayes's⁸. Thus the fate of most of the DDT leaving large adipose stores is unknown. Excretion of DDT through the bile into the faeces has been well demonstrated in the rat¹⁵, but faecal excretion has yet to be studied successfully in man⁸.

Compared with the monkey⁵, dog⁶ and rat⁷, man is unique in the extraordinary slowness with which he unloads fat-stored DDT (Fig. 5). The basis of this species characteristic is not known, but in relation to the magnitude of DDT intake ordinarily confronting him, even man appears to have adequate mechanisms for disposition of the pesticide.

Our highest dose subject (body fat 17 kg)⁹ eliminated fat-stored *pp'* DDT at a maximum rate of 3.1 mg per day and *op'* DDT at 1.1 mg per day. Assuming the same body fat in Hayes's subjects, calculation from the equation fitting their data indicates a maximum elimination of 5.7 mg per day of the DDT isomers combined. Compared with the dietary DDT intake to which Americans may be exposed (0.016 to 0.199 mg per day)^{3,4}, these estimates indicate a reassuring margin of functional reserve that may be called on homeostatically to limit tissue concentrations of the pesticide.

Extrapolating equation (1) down to an approximate mean adipose concentration of DDT in US residents (2 p.p.m.)¹⁶, storage loss from a person having 17 kg body fat would be expected to proceed at an average rate of 19 µg per day (23 µg per day based on Hayes's data). These estimates are similar to measures of urinary DDA excretion in persons having only dietary exposure to DDT¹², and to measurements of dietary DDT intake by US residents in recent years⁴. Presumably, if dietary exposure to DDT were reduced to zero, depletion of fat stores in the human population would proceed from about the same initial rate as now operates in the disposition of dietary DDT.

Setting the integration constant in equation (2) at 0.5284 (indicating an initial adipose *pp'* DDT of 2 p.p.m.), one may conjecture that over a period of one decade at zero intake of DDT, average storage concentration in the general population would decline to 0.3 p.p.m. (0.2 p.p.m., based on Hayes's data). As long as some dietary intake of DDT continues, the decline will, of course, be slower. But this capacity offers some assurance that as dietary DDT intake by US residents continues to fall⁴, excretion will achieve a lowering of body stores as a result.

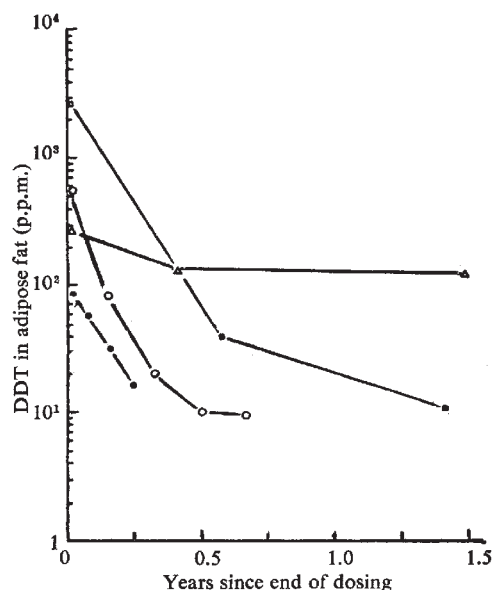


Fig. 5 Loss of fat-stored DDT after dosing in four mammalian species. Δ , In man, after Hayes⁸; $\square$, in monkey, after Durham⁵; $\circ$, in dog, after Deichmann⁷; $\bullet$, in rat, after Datta⁶.

Loss of DDT from fat storage at maximum rates of 4.2 to 5.7 mg per day bears comparison with the 35 mg per day excretion achieved by Hayes's subjects in a steady state of DDT turnover⁸. Direct enteric excretion of the pesticide, with or without metabolic modification, no doubt accounts for the broad difference. A capacity for conversion of DDT to excretable, rather than storable, metabolites could well be critical in promoting excretion, whether the chemical arrives in the gut by ingestion or by mobilization from lipid storage. Gut bacteria can, and probably do, accomplish such conversion¹⁷. In view of the high rates at which ingested DDT can be excreted, it is reasonable to hypothesize that elimination of stored DDT is actually limited by the rate at which it is supplied to the gut, that is, by biliary transport.

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Received February 11; revised March 26, 1972.

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Gravitational Separation of X and Y Spermatozoa

ATTEMPTS to separate the X and Y species of mammalian spermatozoa by such methods as sedimentation and centrifugation in which differences in size or density should be significant have been largely unsuccessful¹. Here I shall develop a simple theory of the motion of spermatozoa under gravity which goes some way to account for these failures. At the same time the theory supports the suggestions² that the slightly greater proportion³ of male to female births in human beings (106 : 100) may in part be due to preferential progress of the lighter Y sperm through the female reproductive tract.

At low sperm concentrations ($< 10^8$ – 10^9 cm⁻³), when interactions between organisms are negligible, gravity produces two effects on each organism. First, there is the downward sedimentation velocity; my measurements on immobilized spermatozoa in normal semen show that this is about $0.3 \mu\text{m s}^{-1}$ at 24°C for both human and bull sperm. Second, a settling object experiences a torque which depends on its shape; immotile sperm tend to orientate head downwards at a rate given by

$$(d\theta/dt) = -\beta \sin \theta$$

where θ is the angle between the long axis of the organism and the vertical⁴ and β is a constant defined by the above equation. Both bull and human sperm have values of β of about $1.5 \cdot 10^{-2}$ rad s⁻¹ if the tail is straight, while curvature of the tail increases β by up to three times this figure. Swimming spermatozoa tend to swim downwards because of gravitational orientation. I have found that motile human sperm, introduced into the bottom of a vertical tube (1 mm internal diameter) containing seminal plasma from which spermatozoa have been removed by centrifugation, rarely reach a height of 2 cm even after several hours. In contrast, sperm liberated at the top of the tube travel about 2 cm downwards in 1 h. These observations cannot be explained by sedimentation alone because inactive or slowly moving sperm remain at the top of the tube; thus active downward swimming under gravity (positive geotaxis) is of considerable importance in spermatozoan motion.

The movements of spermatozoa can be described as a random walk process in which they swim along straight paths for a mean distance λ . If organisms are injected as a thin horizontal layer into a vertical fluid column and then change direction, at random, every τ seconds, the resulting distribution, at time t , is given (if $t \gg \tau$) by

$$n(x, t) = N_0 \left(\frac{3\tau}{2\pi\lambda^2 t} \right)^{1/2} \exp \left(-\frac{3\tau x^2}{2\lambda^2 t} \right) dx$$

where x is the distance along the tube from the plane of injection and N_0 is the total number of organisms⁵. The r.m.s. displacement, d , is

$$d = (\lambda^2 t / 3\tau)^{1/2} = (u^2 \tau t / 3)^{1/2}$$

where u is the swimming velocity. Geotaxis causes a general displacement of the distribution down the tube; if path curvature is small, the extra distance moved downwards due to gravity is $(1/2)\lambda\beta\tau \sin^2 \theta$, and the mean distance, averaged over

Table 1 Displacement of Spermatozoa

Distances (cm)	1 min	1 h	3 h
Sedimentation	-0.0018	-0.11	-0.32
Active downward swimming	-0.03	-1.8	-5.4
Displacement of centre of distribution	-0.0318	-1.91	-5.72
R.m.s. displacement	± 0.071	± 0.55	± 0.95

The calculated displacement of spermatozoa due to random walk movement (characterized by the r.m.s. displacement), sedimentation and active downward swimming when they are introduced into a vertical liquid column (upward displacements being taken as positive).

all directions, is $(1/3)\lambda\beta\tau$. Thus the centre of the distribution moves downwards at a velocity

$$W = v + (1/3)\lambda\beta \quad (1)$$

and the r.m.s. displacement will be virtually unchanged. Table 1 shows the calculated distances travelled from the plane of injection caused by random walk movement (represented by d), sedimentation and geotaxis. Parameters used in the calculations are: $u = 50 \mu\text{m s}^{-1}$ (for human spermatozoa⁶), $v = 0.3 \mu\text{m s}^{-1}$ and $\tau = 10 \text{ s}$. When the centre of the distribution reaches the bottom of the column an equilibrium condition is reached where the upward diffusive motion is just balanced by the downward drift under gravity; the number distribution then decreases exponentially with increasing height with a scale height⁴ of (u/β) .

Table 2 Swimming Ability of Spermatozoa

Time	10 min	15 min	30 min	1 h	2 h
$N(0.5 \text{ cm}, t)$	0.020%	0.025%	0.020%	0.003%	$2.10^{-5}\%$
$R(0.5 \text{ cm}, t)$	1.10	1.12	1.18	1.32	1.64

The calculated percentage of spermatozoa (N) to swim more than 0.5 cm above the plane of injection in a vertical tube and the ratio of Y to X sperm in this region as a function of time.

This description remains valid under gentle centrifugation because both sedimentation and active downward drift velocities are proportional to the effective acceleration⁴. At high rotation rates, however, equation (1) is not applicable, because organisms become completely orientated, head downwards, within the time τ . At 200g complete orientation occurs in less than 0.5 s, and spermatozoa swim directly down the tube. This explains the observation⁷ that rabbit sperm separate into two distinct populations on centrifugation, one of which consists of actively swimming and the other of inactive spermatozoa.

Human X sperm contain, on average, 3 or 4% more DNA than Y sperm⁸. This is equivalent to a 1% difference in head

radius, and calculations show that the resulting differences in swimming velocity, sedimentation velocity and orientation parameter (β) are 0.15% (ref. 9), 1% and 2% (ref. 4), respectively.

Several schemes have been proposed for separating the two types of spermatozoa. In the sedimentation experiments¹⁰⁻¹², where immobilized spermatozoa are allowed to fall down fluid columns, X sperm should reach the bottom first. I have found that the measured variation in sedimentation velocity in a given sample is at least $\pm 20\%$, and is consistent with documented variations in head size and tail length¹³. This is so much larger than the 1% difference between the two types of sperm that very little separation can occur. In addition sedimentation velocities 100 times greater than normal are recorded in these experiments¹¹, suggesting that organisms fall down the column in hydrodynamically linked agglomerations with no possibility of obtaining separation. Similar criticisms apply to kinetic¹⁴ and isopyknic centrifugation¹⁵ techniques, and it is unlikely that gravitational methods will ever produce sufficient separation to permit accurate predetermination of the sex of offspring.

Table 3 Height Attained by Spermatozoa

y (cm)	0	1	2	3
$N(y, \infty)$	100%	0.25%	$6 \cdot 10^{-4}\%$	$2 \cdot 10^{-6}\%$
$R(y, \infty)$	1.00	1.13	1.27	1.43
$r(y, \infty)$	0.98	1.11	1.24	1.40

The percentage of spermatozoa (N) found above a vertical height y from the bottom of the tube, and the ratio R , when equilibrium has been attained. r is the ratio of spermatozoan types at the various heights.

Some degree of separation will occur naturally in suspensions of motile spermatozoa. If equal numbers of Y and X sperm, with identical swimming velocities and with orientation parameters β and $(\beta + \Delta\beta)$, respectively, are introduced into a vertical fluid column, the two types of sperm will progress down the column at slightly different rates. The numbers having reached height h above the plane of injection will initially increase, reach a maximum (at time $3h/u\tau\beta$) and then fall; the ratio, R , of Y to X sperm in this region will increase continuously (Table 2). X sperm will predominate below the centre of the distribution. Finally, in the steady state the ratio will be

$$R(y, \infty) \sim \exp(y\Delta\beta/u)$$

and the calculated variation with height y (measured upward from the bottom of the column) is shown in Table 3. Thus excesses of both Y and X sperm are found in falling distributions, whereas in the steady state Y sperm predominate over most of the volume of the suspension. Stationary or slowly moving organisms will reduce the effective separation. The effects of different values of u , β and τ are shown in Table 4.

Table 4 Effects of Varying Parameters, u , β and τ

$u (\times 10^4) \mu\text{m s}^{-1}$	50	50	50	70	30	50	50
$\beta (\times 10^2) \text{ rad s}^{-1}$	3	3	3	3	3	2	4
$\tau \text{ s}$	10	5	15	10	10	10	10
$N(0.5 \text{ cm}, 15 \text{ min})$	0.025%	0.01%	0.02%	0.16%	$3 \cdot 10^{-4}\%$	0.17%	0.007%
$R(0.5 \text{ cm}, 15 \text{ min})$	1.12	1.09	1.15	1.10	1.17	1.07	1.19

The effects on N and R of varying the parameters u , β and τ for spermatozoa which swim more than 0.5 cm above the plane of injection within 15 min.

Clearly, Y sperm always swim upwards in greater numbers than X sperm.

Gravitational separation cannot occur in the oviducts, where spermatozoa are strongly flow orientated in the ciliary currents^{16,17}, but it may occur in the uterus. Active movements of the tract, which are mainly responsible for spermatozoan transport¹⁸, stir the uterine fluid and tend to reduce separation. Nevertheless, the rapidity with which a significant excess of Y sperm can develop (15 min in low viscosity media) suggests that Y sperm may reach the oviducts in slightly greater numbers than X sperm. The entry of sperm into the uterus is facilitated when a seminal pool forms in the posterior fornix; spermatozoa then have to travel upwards, on average, to reach the uterotubal junction, and this may also aid separation. Finally, if the efficiency of the transport system increases as ovulation approaches, the probability of conceiving a boy will be slightly greater when insemination occurs early in the cycle, and James¹⁹ has recently shown that several characteristic variations in the sex ratio in man can be understood if this latter hypothesis is correct.

I thank the Central Research Fund of the University of London for an equipment grant.

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Received February 2; revised April 5, 1972.

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Reduced Monoamine Oxidase Activity in Blood Platelets from Schizophrenic Patients

AN indirect estimate of monoamine oxidase (MAO) activity based on the formation of ¹⁴C-5-hydroxyindoleacetic acid from intravenously administered ¹⁴C-serotonin revealed no differences between schizophrenic patients and controls¹. The important regulatory functions of monoamine oxidase in biogenic amine metabolism², together with the evidence that MAO-inhibiting drugs may on occasion contribute to exacerbations of psychotic behaviour³, suggested, however, that direct examination of cellular MAO in schizophrenic patients might be of interest.

As mitochondrial MAO activity in human platelets has some similarities in substrate and inhibitor characteristics to MAO activity in brain and other tissues⁴⁻⁷, platelets were isolated from fasting venous blood samples⁸, and MAO activity was

determined by the deamination of tryptamine 2-¹⁴C-HCl according to the method of Wurtman and Axelrod⁹. All assays and data analysis were done by persons unaware of the subjects' diagnoses. A previous study⁷ revealed method and sample variation (calculated as the difference between the paired samples divided by their mean) of $\pm 6\%$ for duplicate samples from the same blood specimen and $\pm 17\%$ for samples from the same individual studied one week apart.

Table 1 Monoamine Oxidase Activity in Schizophrenic Patients and Controls matched for Age and Sex

	Normal controls (n=22)	Chronic schizophrenic individuals (n=33)
Blood platelet MAO activity (nm/mg pro- tein/h)	6.35 $\pm$ 0.86	2.69 $\pm$ 0.40 *
Age (yr)	27.8 $\pm$ 2.5	27.9 $\pm$ 1.8
Sex	18 M, 4 F	29 M, 4 F

* Mean $\pm$ s.e.; $P < 0.001$.

As indicated in Table 1, platelet MAO activity in 33 schizophrenic patients was markedly decreased ($P < 0.001$) when compared with the enzyme activity in 22 non-hospitalized age- and sex-matched normals. Many of the schizophrenic patients had MAO concentrations as low as those observed in depressed patients receiving MAO-inhibiting drugs. Patients were selected for this study only after agreement by three psychiatrists that the diagnosis was unequivocally chronic schizophrenia [according to the criteria of *DSM-II*¹⁰], and thus the results may reflect a bias towards a more severe and chronic patient group. An attempt to rank patients according to severity and chronicity, as well as division of the patients into paranoid, catatonic and chronic undifferentiated subtypes, did not, however, yield any suggestive correlations with MAO activity among these different groups.

Other biological differences between schizophrenic individuals and normals have been found to result from the effects of phenothiazine drug treatment, chronic hospitalization and differences in diet and activity¹¹. We have attempted to evaluate these factors, but a more comprehensive study will be necessary to define fully their possible effects on platelet MAO activity.

Although chlorpromazine and other phenothiazines do not affect MAO activity in animal studies¹², the possibility that the phenothiazine drugs (trifluoperazine, perphenazine and chlorpromazine) received by the schizophrenic patients might be related to their reduced platelet MAO levels was explored by studying 17 chronic schizophrenic patients who had been withdrawn for two weeks or longer (an average of 30 days) from phenothiazines and other drugs. As the mean platelet MAO activity was slightly lower in these patients off drugs (2.63 $\pm$ 0.61 nm/mg protein/h, Fig. 1), and as the life span of platelets is 9.9 days¹³, it seems unlikely that the reduced MAO activity in the schizophrenic patients represents a phenothiazine effect. Furthermore, five patients who were studied within two weeks of admission for acute, first-episode schizophreniform symptoms and who had not received phenothiazine drugs also had significantly reduced platelet MAO activity (3.72 $\pm$ 0.86, $P < 0.05$) compared with the controls.

Lower MAO activity in this small group of five acutely psychotic patients (despite the uncertainties of the diagnosis of schizophrenia in acute first-episode individuals) tends to suggest that the reduction in MAO activity is not an effect of chronic hospitalization alone. Furthermore, 34 other hospitalized psychiatric patients with depression were found in a previous study⁷ to have platelet MAO activities (6.92 $\pm$ 0.48) no different from those of 52 normals [who had mean MAO levels of 6.21 $\pm$ 0.36 which were very similar to our normal

controls] (see Fig. 1). Twenty-three bipolar manic-depressive patients, however, had MAO values (3.65 ± 0.46) intermediate between those of the chronic schizophrenic patients and normals⁷, suggesting the possibility of some common factor affecting platelet MAO activity in both patient groups.

To evaluate the possibility that a reversible inhibitor of monoamine oxidase was contributing to the reduced MAO activity in the schizophrenic patients, dialysis of platelet samples from 10 schizophrenic patients and 7 controls was carried out in

inhibiting drugs in normals and in medical and psychiatric patients has been associated in some instances with the exacerbation of psychotic phenomena, as well as the *de novo* onset of psychosis and lesser changes in mood and activity^{3,17,18}. Similarly, the development of psychotic symptomatology in schizophrenic patients during methionine and tryptophan administration may be potentiated by MAO-inhibiting drugs^{19,20}.

We thank C. Donnelly for technical assistance.

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Received March 24, 1972.

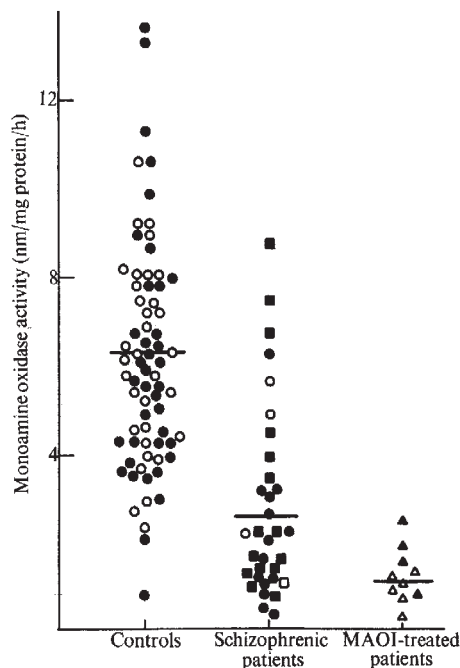


Fig. 1 MAO values for the 33 schizophrenic patients, the combined 75 normal controls, and 10 depressed patients receiving monoamine oxidase-inhibiting (MAOI) drugs (phenelzine, 30–60 mg/day, 8 patients; tranylcypromine, 30 mg/day, 2 patients). ○, Females; ●, males; □ or ■, female or male schizophrenic patients receiving phenothiazine drugs. The platelet samples were frozen and thawed twice before incubation for 20 min at 37° C with tryptamine-2-¹⁴C HCl (8×10^{-5} M, 8.9 mCi/mM) in 0.5 ml. 0.5 M phosphate buffer, pH 7.2 (ref. 11). Product formation was linear with time and enzyme concentration. While variable extraction of the aldehyde and acid metabolites into acidified toluene has been suggested to be a problem with the MAO assay used, this difficulty apparently occurs only when aldehyde dehydrogenase levels are variable or absent as in solubilized MAO preparations²¹. In crude tissue preparations similar difficulties have not been found²², and the addition of aldehyde dehydrogenase from erythrocytes to the platelet preparations in the present experiment did not increase the amount of ¹⁴C-labelled metabolites extracted.

Tris buffer (0.01 M, pH 8.6) for 8 h. No significant difference in activity resulting from dialysis was found for schizophrenic patients or controls.

There is no evidence to suggest that the large hormonal changes which can alter MAO activity in animals¹⁴ are present in schizophrenic patients, nor are there known alterations in biogenic amine metabolism in schizophrenic patients which might be associated with changes in monoamine oxidase. Behaviourally-induced changes in MAO activity in animals have been described^{15,16}, but the possibility of similar changes occurring in human platelet MAO has not been explored.

Whatever the basis for the reduced platelet MAO activity, the possibility that there is a similar reduction of this enzyme in other tissues, including brain, in schizophrenic patients, would be important to explore, for treatment with MAO-

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Genetic Studies of Sleep in Mice

MANY alterations of the central nervous system have been discovered in mutant strains of mice, usually by neurological, behavioural, biochemical or anatomopathological investigation^{1–3}. Here we report that the polygraphic study of the states of sleep in chronically implanted mice is also a very sensitive index for examining the functioning of the central nervous system in the inbred strains.

We have studied forty 12–15 week old, male mice (*Mus musculus*) from two inbred strains (C57BR/cd/Orl, CBA/Orl) and their F₁ hybrids (C57 × CBA, CBA × C57). Under pentobarbital anaesthesia, four electrodes were chronically implanted on the fronto-parietal cortices and two electrodes were fixed in the neck muscles. Animals were housed individually in glass jars on synthetic bedding with unlimited food and water. Room temperature was constant: 24° C ± 1° C. Lighting schedule consisted of 12 h light (0700–1900) and 12 h dark (1900–0700). Fourteen days after the implantation, ten groups of animals were simultaneously and continuously recorded on

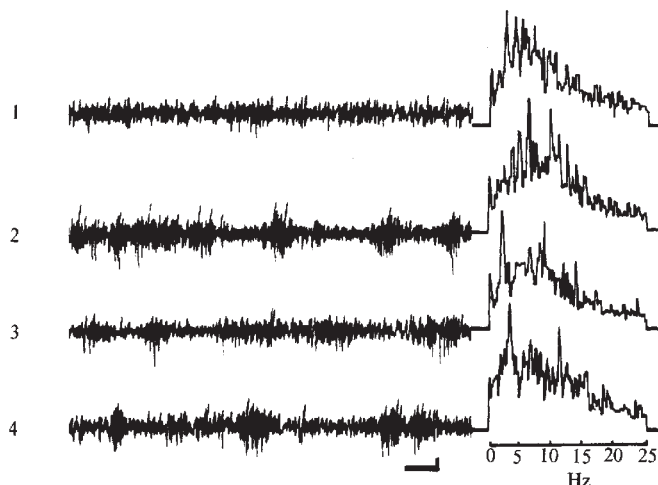


Fig. 1 Left, samples of cortical activity during slow wave sleep (calibration 3 s, 50 μ V) in 1, C57BR; 2, CBA; and F_1 hybrids: 3, C57 $\times$ CBA; 4, CBA $\times$ C57. Right, spectral analysis of the same samples. Note the spindling and the peak of frequency at 10–12 Hz in both CBA and hybrids.

'Alvar' polygraphs for an 8-day period. The cortical activity was also recorded on an 'Ampex' magnetic tape for subsequent spectral analysis of the electroencephalogram (EEG) by ATREEG computer (Thomson-CSF). The quantities of sleep and waking states were determined by visual analysis every 30 s.

Our results clearly revealed both qualitative and quantitative differences in the EEG patterns during the states of sleep. No such differences were evident during waking.

In the CBA strain, during "slow wave sleep", numerous high amplitude 10–12 Hz cortical spindles were associated with 2–5 Hz slow waves, while only 2–6 Hz slow waves occurred without any spindles in the C57BR strain (Fig. 1).

The quantitative analysis of the duration of the states of sleep showed only very small interindividual variations in each strain of mice. The duration of daily total sleep in the CBA (791 ± 9 min; $n=35$) was significantly greater than for the

C57BR (705 ± 10 min; $n=35$) ($P<0.001$). The total duration of paradoxical sleep was more important in C57BR (85 ± 3 min) than in CBA (60 ± 2 min) ($P<0.001$). The ratio between paradoxical sleep and slow wave sleep was 12% in C57BR and 7.6% in CBA.

The circadian rhythm of the sleep-waking cycle was also different (Fig. 2). The difference in total sleep time during day and night was relatively small in the CBA strain while it was much greater in the C57BR.

The study of F_1 hybrids demonstrated that the quantitative aspects of sleep of crossing both C57 $\times$ CBA and CBA $\times$ C57 were not statistically different. The pattern of the electrocortical activity (spindles associated with slow waves), the quantity of sleep and its variation during day and night were similar to the CBA strain.

The spindle activity which occurs during physiological sleep is thought to be generated in the thalamus and/or in the cortex^{4,5}. The absence of any spindling in C57BR reflects a probable anatomical or functional alteration of these structures, and deserves further investigation. The CBA strain has retinal degeneration (rd), a recessive mutation which induces degeneration of photoreceptor cells, but leaving ganglion and bipolar layers intact^{6,7}.

This relative blindness might explain the poor circadian alteration of sleep between day and night in the strain. Hybrids which have no retinal degeneration, however, show the same circadian sleep organization as the CBA. Thus, it is more likely that genetic factors are involved, not only in the occurrence of spindle activity, but also in the organization of the sleep cycle.

In conclusion, the polygraphic quantitative study of sleep in mice shows functional alterations of the central nervous system and may be of considerable importance in the analysis of the mechanisms of both slow wave and paradoxical sleep.

The spectral analysis was done at the CRSSA (Psychology Department).

This research was supported by grants from DRME, INSERM and CNRS.

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Received January 17, 1972.

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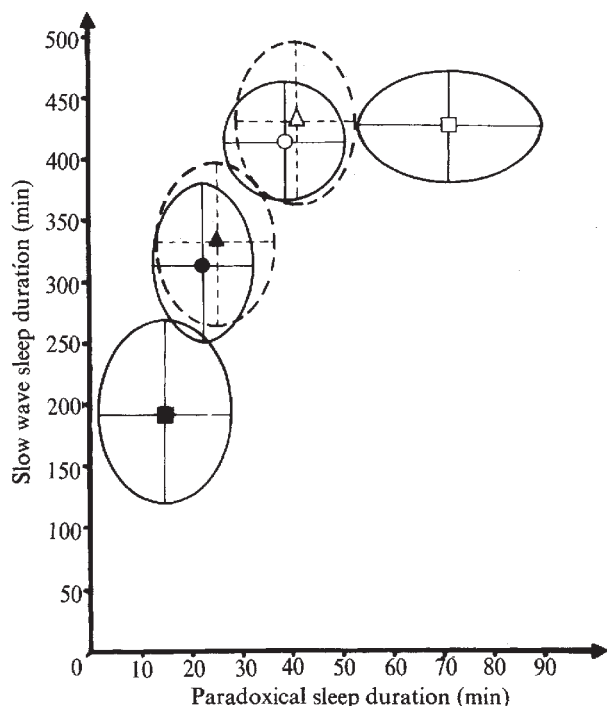


Fig. 2 Ordinate: duration of slow wave sleep (min). Abscissa: duration of paradoxical sleep (min) during day (white) and night (black). ■□, C57BR; ●○, CBA; ▲△, hybrids. Results are expressed in mean ± 2 s.d.

Visual-Auditory Distance Constancy

Engel and Dougherty's report¹ on visual-auditory distance constancy states that judged simultaneity of equidistant flashes and clicks varies less as observer-source distance changes than would be predicted from variation in the arrival of clicks at the ears. That is, apparent simultaneity of visual and auditory stimuli exhibits a degree of perceptual constancy with variation in their temporal spacing concomitant on source distance. While this particular instance of auditory constancy has not been noted previously, early work by Mohrmann² suggests an explanation of the recent observation. Mohrmann required his subjects to judge the loudness of a variety of

sounds including speech, music, metronome clicks, noise and tones located at 0.75, 2.37 and 7.50 m. Although the intensity of the sounds at the ear varied with their distance, loudness changed less than predicted from the stimulus. This auditory loudness constancy occurred either in the dark without prior acquaintance with the laboratory, or with blindfolded subjects who viewed the laboratory between tests, or with subjects who viewed the laboratory throughout. Thus, even without visual information, the loudness of sounds is relatively constant as stimulus intensity varies with source distance. It is conceivable that in the Engel and Dougherty experiment the constancy of apparent flash-click simultaneity was secondary to the loudness constancy of the click. That is, constant loudness may have provided a basis for judgments of stimulus timing. Such an interpretation seems more plausible than one attributing both forms of constancy to a common base, as loudness constancy is probably correlated with the streaming velocity of the sound³. It would be of interest to compare the degree of auditory loudness constancy with that of flash-click constancy at different distances to establish whether the functions are similar.

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Received December 16, 1971.

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Reply to Engel and Dougherty

WHILE examining the results found by Engel and Dougherty in their investigations of visual-auditory distance constancy¹, we noticed a simpler explanation than the one which they suggest. Broadly, their results (ref. 1, Fig. 1) lie parallel to the line which would apply if no account were taken of propagation time, but 50 ms lower. This suggests that, while observers have the ability to assess relative time of arrival of the stimuli, they have not developed the skill for interpreting this information; 50 ms, corresponding to 17 m, could be some sort of average based on the relative delays met in everyday life.

The suggested lack of skill is borne out by the experience of those who watch cricket only rarely. The delay in the click of bat hitting ball always comes as a slight surprise. With reference to even larger distances, many people do not realize that the flash of lightning and the crack of thunder originate simultaneously: usually, these things just do not matter to us. The acquired intellectual skill of those who count off the seconds as thirds of a kilometre, or fifths of a mile, is, of course, a different matter altogether.

Calculation of the optimal auditory-visual delay for a large cinema could, in principle, be a very complex matter, according to whether each member of the audience is considered as seated in the theatre and seeing either a picture on a screen or a view through a window, or as seated at the position of the original camera. The fact that no extensive study seems ever to have been made, or needed, is further evidence of the fortunate fact that people are generally uncritical on this matter.

The objection to our suggestion lies in the measurements shown at a very short distance in Fig. 1 of ref. 1. Although we cannot explain this result, we can quote other data which suggest that it should be treated with reserve. The matter has been of interest in telecommunications, because the sound and vision signals in television may be sent by different means, possibly with markedly different propagation times. Three separate investigations, using differing methods, have led to the general conclusion² that a fairly wide tolerance is permissible, equal to a range of about ± 100 ms referred, not to zero, but to a delay of sound relative to vision in the vicinity of

35 ms. This result, obtained at distances around 2 m, gives a data point at +2, -35 on Fig. 1 (of ref. 1), which lies conveniently close to a line projected from the larger distances.

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Received December 29, 1971.

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Mortality of Vultures caused by Electrocution

IN the south-western Transvaal in South Africa, Cape vultures, *Gyps coprotheres*, have been accidentally electrocuted by contact with 88 kV high tension power lines; it is possible that, occasionally, individuals of other vulture species may be involved as well. It is not known whether these large birds are killed trying to perch or when flying off, or both.

Table 1 Minimum Numbers of Vultures Electrocuted 1970-71 *

Month	1970	1971	Total
January	1	4	5
February	4	3	7
March	10	16	26
April	5	33	38
May	3	11	14
June	3	2	5
July	0	2	2
August	0	0	0
September	0	0	0
October	0	2	2
November	2	8	10
December	7	13	20
Totals	35	94	129

* Figures for early 1972 are: January, 5; February, 4; March, 10.

From January 1, 1970, to March 31, 1972, at least 148 vultures were killed. Table 1 shows that there is a quiet period at the time of year when *G. coprotheres* has eggs and young in the nest in the Transvaal. Presumably the concentration of vultures in an area at any one time would depend on the amount of food available. The figure 148 relates to the number of bodies found during inspections, frequently carried out to determine the cause of line faults: vultures have proved to be troublesome in this regard. As the power lines pass through both densely populated areas of human habitation and rural scrub country, additional corpses could easily have been removed by man or scavengers, disappeared as a result of decomposition, or have been overlooked. Thus the actual number of deaths was no doubt greater.

It would be of value to know if vultures are also electrocuted in other areas, particularly those where there are relatively few large trees for them to perch in, as is the case in the SW Transvaal. Birds with a low reproductive rate, like vultures, usually have a correspondingly low death rate, and accidental electrocution on a large scale might have a significant impact on vulture populations.

This report was written while I was visiting the Mammal Research Unit of the South African Council for Scientific and Industrial Research, supported by a CSIR travel grant.

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BOOK REVIEWS

The Fourth Law of Thermodynamics

Thermodynamic Theory of Structure, Stability and Fluctuations. By P. Glansdorff and I. Prigogine. Pp. xxiii+306. (Wiley: New York and London, June 1971.) £5.50.

"If the reader is a good boy and eats his meal, he may have his pudding" might have been said by Professor Glansdorff to Professor Prigogine, as they firmly placed the newest and most stimulating part of this book at the end. The end, that is Part 3, is devoted to chemical instabilities: there are discussions of the oscillations in time around the steady state which can occur in chemical reactions; and of chemical instabilities which can lead to periodic structures in space. The latter are illustrated by some fine photographs of test tubes showing the Zhabotinsky reaction. There is, furthermore, a discussion of excitable biological membranes. Such membranes may, for some purposes, be considered as having two main states: the depolarized state, and a polarized state which is characterized by different ionic concentrations on the two sides. The transition between the two states is likened to a phase transition.

The space organization brought about by the symmetry-breaking instabilities can lead to the so-called dissipative structures. These are open systems which maintain themselves by the appropriate utilization of energy and/or matter exchanged with the surroundings. This suggests that dissipation can be a source of order in space and/or time.

In this part of the book the bridge-building between physics and biology is clearly being continued. It has been making all too slow progress these last twenty years or so, but is now showing signs of new buds and quickening interest¹⁻³. Biologists who can understand the mathematical details of the first faltering steps of the physicists in this direction are still only a small minority, so the evaluation of these contributions will take time. Yet this is precisely what is urgently needed: not only must biologists come forward to evaluate, they are needed to stimulate and to help give direction to the next steps. The cleavage which has often occurred in economics between mathematicians who are doing "lovely mathematics" and the economists who want useful models can, and should, be avoided.

In the book under review, the mathematical analysis of the phenomena described in Part 3 depends in all instances on the general theory, developed in Part 1. This is the main meal (presented in nine courses). It starts off in the only way yet devised⁴ of approaching the subject, namely by discussing balance and conservation equations for energy, mass and momentum. In this respect it resembles the well-known books by de Groot and de Groot and Mazur^{5,6} and Prigogine's own earlier book⁷. But the new book soon strikes out largely on its own, following the series of papers published by the authors and their colleagues since 1954. Of course, there is some overlap, in particular in the treatment of the chemical affinity, defined earlier in the book in terms of chemical potentials μ_γ of components γ participating in a reaction with stoichiometric coefficients ν_γ by

$$A = - \sum_{\gamma} \nu_{\gamma} \mu_{\gamma} \quad (1)$$

On page 39 the affinity appears again in the relation with the equilibrium constant K

$$A = RT \ln (K c_1^{\nu_1} c_2^{\nu_2} \dots) \quad (2)$$

The connexion between these two results is made *via* the perfect gases (it applies also to ideal solutions), and it is a pity that, in view of the importance of chemical reactions to the philosophy of the book, a more general account⁸ has not been included in Part 1.

Linear thermodynamics, minimum entropy production and the Onsager relations are not the main matter of interest and are passed over rapidly. The main meat of Part 1 resides in the discussion of the stability of departures from non-equilibrium states. The theory is expected to apply only for small departures from such reference states as one may select and this restriction is mentioned explicitly. This must firmly be borne in mind even when reading the authors' more ebullient passages. For equilibrium states the authors have little to add to the well-known conditions of stability ($c_v > 0$, $K_T > 0$).

It is a notable success that the theory can handle both thermodynamic and hydrodynamic stability questions. This is achieved by passing from the entropy s per

unit mass to the quantity

$$z \equiv s - \frac{1}{2} T_0^{-1} (v)^2 \quad (3)$$

where v is the average macroscopic velocity and T_0 the temperature in the reference state (for example, at the "beginning" of a fluctuation). This enables the book to include a Part 2, devoted to hydrodynamic applications, which greatly enhances its interest.

The book deserves to be judged by the highest standards, and in view of this the exposition has some shortcomings. It is surprising that the authors find the number of independent variables as one number when extensive quantities are reduced to intensive quantities by taking them per unit mass, and another number when they are reduced by taking them per unit volume. This could be connected with the neglect of the normalizing condition $\sum N_\gamma = 1$, when counting independent variables.

In (6.37) the authors derive a condition $[\partial_z \delta_m^2(\rho z) \geq 0]$ for stability which is claimed to be both necessary and sufficient. At first sight this seems puzzling because this condition is compatible with stability-violating relations $[\omega_r > 0 \text{ and } \delta_m^2(\rho z) > 0]$ for a mode. But the authors evidently mean their condition always to be taken together with the condition $\delta_m^2(\rho z) < 0$. The condition which assigns the molar mass M_r to a position which should be taken by an extensive variable, according to equation (1.4), is surprising, but causes no serious trouble. The argument at the bottom of page 59 is not valid; the product of two symmetric matrices need not be symmetric, unless the matrices commute. The authors clearly mean that the roots of a matrix are real if this matrix is obtained by multiplying two symmetric matrices. There are also some irksome adjustments which the reader has to make for himself, but which would have been worthy of a comment (or should have been eliminated) as, for example, the change of sign in the entropy flow Φ on page 53 compared with its first introduction on page 1, or when the volume integral in the expression for the entropy production is omitted on pages 60 and 113. This latter change is not serious because the reaction considered is homogeneous, but it still causes dimensional

inconsistencies. The main lines of the book are clear enough, however, and the contents exciting enough for readers to accept the odd error or infelicity without being unduly perturbed.

The main warning to potential readers is that behind the deceptively simple mathematics lie concepts and assumptions whose precise ranges of validity are not yet known, and have only recently been studied. This may account in part for the occasional lack of lucidity in the book which may give rise to some discussion⁹ within the next few years.

We now take the opportunity to ask: what are the special elements in thermodynamics with the extrapolatory power which enables one to give thermodynamic discussions of non-equilibrium situations? Different answers may be given by different workers in the field¹⁰. Meixner and his school may say, for example, that a non-equilibrium entropy cannot be defined. My own answer is perhaps more optimistic: I would affirm that one needs two notions to see the possibilities of extrapolation. The "first notion" is the "basic trick"¹¹ of thermodynamics which enables a system (which may have gradients of temperature and so on built into it) to be considered as composed of elements to each of which normal thermodynamics applies. Each element must be large enough for this purpose, yet small enough for the gradients to be unimportant within each element. The state of the whole system need not be an equilibrium state for neighbouring elements may differ in temperature, and so forth. Such a state may be called a reference state "O"—this connects with the terminology of Glansdorff and Prigogine. They rightly emphasize that they assume throughout *stable local equilibrium*. Its meaning can be explained in two parts. The stability part of it is expressed by 4a (below), while the local equilibrium part of it means that, within each small mass element, the local entropy s is the same function of the local macroscopic variables as at equilibrium*. Their treatment incorporates therefore a specific application of the basic trick.

The "local equilibrium" part of the "local stable equilibrium" assumption is enough to make the usual thermodynamic relations available, except that it is preferable to express extensive variables (like entropy S) in intensive form (s) by considering them, for example, per unit mass. For *small* departures from a *stable* reference state (denoted by the suffix O) the following local thermodynamic conditions hold

$$\delta^2 s \equiv \sum_{r,u} \frac{\partial^2 s}{\partial x_r \partial x_u} (x_r - x_{rO}) (x_u - x_{uO}) \leq 0 \quad (4a)$$

* This does not mean that each element can be thought of as in fact in equilibrium or in any sense at its maximum entropy value.

$$\text{and } \partial_t \delta^2 s \equiv \frac{\partial}{\partial t} (\delta^2 s) \geq 0 \quad (4b)$$

Here the x_r are a specific set of independent thermodynamic variables (energy and volume per unit mass and the mass fractions of the various components), and the left-hand sides introduce the notation used by the authors. We shall now discuss two cases separately. Case A: if the reference state is an isolated equilibrium state, then the additional relation

$$\delta s \equiv \sum_r \frac{\partial s}{\partial x_r} (x_r - x_{rO}) = 0 \quad (4c)$$

becomes available for infinitely small departures from this state. It expresses the extremum property of the local entropy s . Condition (4a) then sharpens this so that this state is one in which the extremum is actually a maximum. But because (4c) is not part of what is meant by the local stable equilibrium assumption, it does not need to hold in a non-equilibrium reference state which satisfies the local stable equilibrium assumption. The interpretation of (4a) as characterizing an extremum property holds therefore only if the reference state is an equilibrium state. Condition (4b) becomes then the non-negative local entropy production rate $P[s]$. It can be written equivalently as $2 P[s] \geq 0$, where the factor 2 arises (page 66) because $\delta^2 s$ is a quadratic form in the quantities which characterize the departures from the reference state. Case B: but, if the stable reference state is not an equilibrium state, then we have only (4a, b). They state that $\delta^2 s$ increases from its initially negative value with lapse of time so as to recover (eventually) its undisturbed, though non-equilibrium, value. The quantity $\partial_t \delta^2 s$ is then called the "excess" entropy production rate, and $\partial_t (\delta^2 s)$ is no longer equal to $2 P[s]$.

Let us understand clearly what has happened here. In case (A), when the reference state is an equilibrium state, the global form of (4b) is positive by the second law, and the global form of (4a) is deduced as a sufficient stability condition. In case (B), when the reference state is a non-equilibrium state, the authors start with (4a) because it expresses the stability part of their local stable equilibrium assumption, which is basic to the whole book. The expression (4b) has now in general an undetermined sign because the second law makes no assertion about the sign of the excess entropy production. The condition that the sign be again positive thus becomes the authors' sufficient condition for stability. One sees that the local conditions (4a) and (4b) are involved in cases (A) and (B) in an asymmetrical manner. The extent to which condition (4b) in case (B) is either deduced or a new principle is left a little vague it is probably safe to assume that it is intended to be an additional principle in thermodynamics.

With the explanations just given, the procedure is clear enough. A criticism which does arise is a minor one of terminology. The term *local stable equilibrium* assumption has the technical meaning given above. It does not mean that the system is always locally stable, that is, that it satisfies (4a) and (4b); were this assumed the theory would exclude instabilities at the outset, which, of course, it does not. If one wants to retain the authors' terminology as far as possible, one might replace their term by *local semi-stable equilibrium assumption*, in order to express that only one of their two sufficient stability conditions is imposed as a local assumption.

For purposes of further explanation of a point which is perhaps not quite clear from the book, let us now assume that, the question of stabilities having been clarified, a system is truly locally stable, in the sense that (4a) and (4b) are satisfied, and that the boundary conditions for the individual volume elements are consistent with the global boundary conditions. The system is then also globally stable by assumption. With this as a basis, one still has to make one more assumption to obtain a mathematical expression of this statement: the summation of corresponding local extensive variables over the whole system (near the reference state) gives the value of the corresponding global variable. Given this assumption, the *global conditions for the stability of the reference state* are for fixed boundary conditions immediately satisfied by summation of (4a, b)

$$\delta^2 S \leq 0, \quad \partial_t (\delta^2 S) \geq 0 \quad (5)$$

With S appropriately expressed in terms of other variables, one can now express such conditions thermodynamically in a variety of ways, and trace the consequences of their failure (which should result in instability).

Glansdorff and Prigogine base their analysis on (5), and, along with most authors, they adopt the assumption just outlined. Without this assumption there could, of course, still be a theory of fluctuation and stability, but it would not be thermodynamic in character. It represents therefore the second notion needed, and both are essential for a thermodynamic theory of this kind. The analyses given in this book are not always thermodynamic in character, but contain also normal mode stability analysis (perhaps one might call them stochastic methods). Part III is in fact largely based on such an approach, and it could be argued that the results there are not in the main thermodynamic in character.

It is fortunate that the second notion actually "works" in that the results obtained from it cohere with other theories and experiments, as is seen so well in this book. If an assumption works and is important enough, it may be called

a "law", and it has appeared to the reviewer that it in fact qualifies as a "Fourth law" of thermodynamics, which is stated more fully elsewhere^{11,12}. There is nothing very startling about it since this law is always implicitly adopted, and people know about it. It is rarely displayed, however, as a "law". If we do so, the following symmetrical situation arises: Zeroth law—empirical temperature exists. First law—internal energy exists. Second law—entropy and absolute temperature exist. Third law—states with $T=0$ do not exist. Fourth law—for a class of non-equilibrium states, and for equilibrium states, extensive and intensive variables exist. The wordings are here intended to bring out the analogies, they are not intended to be complete statements of the laws.

Thus the puzzle of the thermodynamics of non-equilibrium can now be given quite a brief technical answer: *non-equilibrium thermodynamics can be constructed from equilibrium thermodynamics by using the basic trick, and by then appealing to the Fourth law to obtain global properties.*

It is now also possible to describe the contents of this volume with some precision: *It contains theory and applications of thermodynamics as applied to small fluctuations from reference states which are subject to the local stable equilibrium assumptions.* The methods and results may be valid beyond this range¹³; but this is not known for certain so the description given is the least claim that can safely be made. The book should certainly be read by a wide variety of persons interested in stability problems in physics, chemistry and biology.

I am grateful to Professor P. Glansdorff for helpful correspondence, and to Professor R. O. Davies for discussion.

P. T. LANDSBERG

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⁴ Prigogine, I., *Etude Thermodynamique des Phénomènes Irréversibles* (Desoër, Liège, 1947).

⁵ de Groot, S. R., *Thermodynamics of Irreversible Processes* (North Holland, Amsterdam, 1951).

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⁷ Prigogine, I., *Introduction to Thermodynamics of Irreversible Processes* (Thomas, C. C., Springfield, Illinois, 1955), third ed. (Wiley, Interscience, New York, 1967).

⁸ Prigogine, I., and Defay, R., *Chemical Thermodynamics*, 83 (Longmans Green, London, 1954).

⁹ Lavenda B. H., *Lett. Nuovo Cimento*, **3**, 385 (1972).

¹⁰ *Proc. Cardiff Conf. on Thermodynamics, 1970* (Butterworth, London, 1970).

¹¹ Landsberg, P. T., *Thermodynamics with Quantum Statistical Illustrations* (Wiley, Interscience, New York, 1961).

¹² Wright, P. G., *Proc. Roy. Soc., A*, **317**, 477 (1970).

¹³ Schlögl, F., *Z. Phys.*, **243**, 303 (1971).

Electrode Kinetics

Kinetics of Electrode Processes. By Tibor Erdey-Gruz. Pp. 482. (Adam Hilger: London, January 1972.) £9.

THE author of this book started work in this subject just over forty years ago when the foundations of the modern approach to electrode kinetics were being laid by Bowden and Rideal, Gurney, Butler, Frumkin and Volmer. The paper published by Erdey-Gruz and Volmer in 1930 is one of the classics of this period. The reader, therefore, expects a treatment which takes a broad and authoritative view. It is difficult to digest the vast mass of material now available in this field of kinetics and to present a coherent picture. Professor Erdey-Gruz has succeeded remarkably well. He points out in his preface that it is impossible not to be selective. His selection can be broadly summarized by recalling Delahay's classification of electrochemists into "Tafel Plotters" and "Laplace Transformers"; this is very much a book for the former and may not please the latter, though it would be useful for them to read the other point of view. The most nearly comparable book is Vetter's *Electrochemical Kinetics* which is nearly twice as big. Erdey-Gruz's book is not so theoretically oriented, tending to be more descriptive, and it has the great advantage, now, of being more up-to-date. A great many references are discussed and especially with the recent ones the style becomes non-committal in the manner of an annual report.

The plan of the book is good: the first three chapters describe the principles of the three important components of electrode reaction: charge transfer, chemical reaction and diffusion. Chapter 4 summarizes results for particular electrodes: hydrogen, oxygen, chlorine, redox electrodes and metal/metal ion electrodes. The remaining three chapters survey molten salts, semiconductors and anodic films. The relegation of double layers and experimental techniques to the appendix could be criticized and the discussion of "absolute reaction rate theory" here is of doubtful value. Some criticism in detail would also be possible, for example the different figures on pages 144 and 174 for the same relationship and the repetition on pages 342/3 and 349/50. But these should not detract from the solid achievement of the whole which should be a standard work for many years.

The translation is excellent.

ROGER PARSONS

Models, Monkeys and Men

Casual Groups of Monkeys and Men: Stochastic Models of Elemental Social Systems. By Joel E. Cohen. Pp. xiii + 175. (Harvard University: Cambridge, Massachusetts; Oxford University: London, December 1971.) £3.75.

WHEN faced with a complex phenomenon, the pessimist (who sometimes calls himself a realist) despairs at the complexity of the situation and is satisfied with a description of as many of the components of the system under consideration as possible. On the other hand, the optimist (who sometimes calls himself a mathematical modeller) sets out to find those aspects of the problem which lend themselves to a relatively simple mathematical formulation with the hope that the more complicated problem can be effectively modelled by a combination of such simple models. Needless to say, this is not always possible or even useful but, unless it is attempted, the underlying simplicity of many systems will never be realized. In this book, Joel Cohen has taken such a phenomenon, the size distribution of casual groups (groups in which individual members are free to enter or leave) and has shown that in a large variety of cases, the size distribution can be accounted for by a relatively simple model. As the title implies the groups range from groups of humans occupying cars on an expressway to sleeping groups of vervet monkeys on the East African savannah.

The model that Cohen uses to describe the various situations is a linear one-step transition (LOST) model, in which the probability of joining or leaving a single subgroup is a linear function of the size of the subgroup. This assumption, of course, ignores all of the complex psychological, sociological and ecological considerations which would be necessary to understand why an individual joined or left a particular subgroup. The most important result in the book is that these considerations do not matter at the level of the available data. While the LOST model is unlikely to be able to predict the behaviour of a single individual for very long, it does very well indeed at predicting the behaviour of a group of individuals each of which is exercising its individuality. Furthermore, the groups from which the subgroups are drawn do not have to be very large in order for the two parameters of the LOST model to effectively describe it.

The book begins by discussing in detail the data that have been collected on subgroups and the various models which have been posed to fit them. These models are all static models in

the sense that the system is assumed to be at an equilibrium so that some distribution such as the Poisson or negative binomial (truncated for the zero class) or some more complicated one can be fitted to the data. Cohen's model, in contrast, is dynamic and is shown to lead to a negative binomial distribution at equilibrium. This allows for the possibility of an independent test of the model. One estimate of the parameters can be based on measurements of the dynamics of joining and leaving subgroups and another based on the distribution of subgroup sizes. Since no suitable data had been taken, Cohen collected his own on play groups of infants at a Cambridge (Massachusetts) nursery school. The strongest impression is how terribly difficult it is to test in a meaningful way a model which is conceptually so simple. This cannot help but suggest even a slightly more complex model would be impossible to test. This should give the most incurable optimist some second thoughts.

This is not an easy book to read. Cohen spares the reader none of the formidable details in the many calculations and derivations; and considerable mathematical sophistication is required to see why he bothers with some of the details to the extent he does. It is not intended to be a book on all aspects of subgroup formation but rather on all aspects of models of subgroup formation and, as such, it is outstanding. One could begin with the LOST model applied to a particular system and go on to look at the different levels which underlie the dynamics of the system, and which could provide more information about the parameters of the LOST model. Cohen gives one example of this in the later chapter, discussing the effect of background noise on maximum size of subgroups. It is clear where to start using the model and what its limitations might be. There is none of the lingering feeling which so often comes when reading about a mathematical model that if it is really such a good model, the author himself should be using it for something.

MONTGOMERY SLATKIN

Tracing Reactions

The Kinetic Isotope Method and its Applications. By M. B. Neiman and D. Gal. Pp. xii + 309. (Elsevier: Amsterdam and London, 1971.) Dfl.85; \$25.

FOLLOWING the appearance of *A Kineticus Izotop Modszar es Alkalmazasai*, published in Budapest, the present English edition, which differs relatively little in scientific content, has now been prepared by Professor Gal.

The underlying principles of the

kinetic isotope method are outlined in some detail in the first four chapters. Here it is shown how tracer techniques can be used to determine, in a complex system, not only the sequence of formation and separate rates of production and destruction of intermediate products but also the order of reaction with respect to each of the participating species.

There then follow examples of applications of the kinetic isotope method to thermal decomposition reactions (chapter 5) and to gaseous oxidation reactions (a subject of special interest to the authors who have done valuable original work in this field) (chapter 6). The next rather brief chapter outlines some applications of isotopic labelling to competitive reactions of free radicals. Chapters 8 and 9 deal respectively with the oxidation (mainly of hydrocarbons) in the liquid phase and with various reactions of hydrocarbons and alcohols taking place at the surface of heterogeneous catalysts. Finally, an account is given (by Dr Laszlo Latzkovits) of the use of isotopic tracer techniques in the study of reactions of biochemical interest. The kinetic isotope method was first used in connexion with this type of system, so this relatively brief chapter is perhaps unable to do justice to the large volume of published work in this field.

The appearance of this book has, by bringing together in a single volume so much relevant data, undoubtedly drawn attention to the wide applicability of the kinetic isotope method. But there is a tendency throughout for the emphasis to be placed on systems in which the authors have a personal interest and indeed, in some places, the text appears to be little beyond a collection of the authors' papers in book form! Although some slant towards work in the USSR and Hungary might reasonably have been expected, insufficient recognition has certainly been given to the large number of important investigations carried out elsewhere. The relatively few references to the work of British and American authors seem to have been arbitrarily chosen and are, in many cases, considerably out of date.

Although then this is a useful reference book and will be of considerable interest to those who have worked in one of the several different fields where the kinetic isotope method has been successfully applied, the treatment is not sufficiently rounded (and incidentally the price is too high!) for the more general reader who wants a well-balanced introduction to the subject. Nevertheless, I hope that by drawing attention to a technique which, despite its wide applicability, has been all too little used, this book will stimulate renewed interest in the kinetic isotope method.

C. F. CULLIS

Mathematical Genetics

Mathematical Topics in Population Genetics. Edited by Ken-ichi Kojima. (Biomathematics Vol. 1.) Pp. ix + 400. (Springer: Berlin and New York, 1970.) 68DM; \$18.70.

THIS book is a collection of research and review papers on mathematical genetics. It is expensive and might have been more useful to the kind of reader for whom it is presumably intended if the price had been brought down by leaving out the research papers.

The review papers vary in the degree of involvement with the subject assumed on the part of the reader. Kojima and Lewontin, for example, give a basic general review of the subject of selection in multi-locus systems, while Kimura's article on diffusion models would be very difficult to follow without a clear understanding of the difference between a limiting distribution of gene frequency and a distribution of number of alleles under mutational flux.

In the first article of the book, Wright describes his theory of evolution by the interaction of random processes and natural selection. This is the only mechanism of evolution consistent with genetics which has been proposed since Darwin, and Wright's article would repay study by non-specialists. Theories of the evolution of dominance are reviewed by Sved and Mayo. They make a useful further contribution to the industry of finding out what Fisher really meant. Turner reviews the current status of Fisher's unfortunately-named Fundamental Theorem of natural selection. His conclusion seems to be that the fundamental fact about selection is that, even if something is maximized, it does not tell us much about biology. Crow reviews the literature on genetic load in a very clear fashion. It is a pity that he does not discuss more fully the objection to the concept of load raised by Sved, Reed and Bodmer, who pointed out that, when there is substantial polymorphism, the fully heterozygous genotype is unlikely to crop up in a finite population. It is therefore of doubtful utility to use it as a standard of fitness. Levins presents a new formulation of the concept of group selection. It is difficult to follow his account, because many of his parameters are left undefined. Incidentally he attributes a theorem of Wright's to Li. It is untrue that, as he states, Fisher's Fundamental Theorem of selection is a weaker statement than Wright's maximization principle.

In summary, this book contains some review papers which may be useful to the non-specialist, but is likely to be bought only by the specialist in this field.

BRIAN CHARLESWORTH

Marine Mammals

Marine Mammals of the Sea. Edited by Sam H. Ridgway. Pp. xiii+812. (Charles C. Thomas: Springfield, Illinois, January 1972.) \$45.

TWELVE contributors have provided ten chapters on widely varying aspects of marine mammals for what will be a useful reference work in certain fields for some years. Some chapters cover the same ground as has been reviewed in recent books and multi-author works such as Pilleri's *Investigations on Cetacea* but as each chapter is written by individuals now active in the field and laboratory there is much new material and original comment. The book seems to have been initiated with the intention of considering the problems involved in maintaining a colony of marine mammals and a few chapters are still basically directed to this end. The short chapter on the central nervous system, for example, considers some practical aspects, indicates some topics of recent research, asks some questions and concludes with 258 references but could hardly be considered a substantial review. Other chapters, however, deal much more exhaustively with a topic and that on comparative microscopic anatomy by J. G. Simpson and M. B. Gardner is full of interesting information and is profusely illustrated with original photomicrographs. Some of these suffer from the inevitable troubles in fixation of material only too often difficult to acquire in a fresh state. In many ways this chapter builds on the previous one, devoted to general anatomy, expands it even to the extent of repetition and on some organs, the stomach for example, gives a more accurate account. These two chapters bring together much information not easily available to those interested in structural modifications in seals and dolphins. The first two chapters are concerned with descriptions of genera and species. These often suffer from being too short and offer cryptic information. There is scant account of growth changes and it would be useful to know from just which particular specimens, and how many, some of the alleged distinguishing characteristics were derived. We doubt whether the classification of the Delphinidae will receive universal approval but as Dr Masaharu Nishiwaki disarmingly points out "certain groups may have to be reclassified". The stippling technique used in the illustrations unfortunately suggests that many if not all dolphins are spotted or speckled, almost morbilliform in appearance, and makes it difficult to discern true markings. Many spelling mistakes could have been removed: *aduncas*, *Legenodelphis*, *crusiger* (who is not a person as implied in Fig. 1-51), *Sotolia*, *Zolophus* and

Zalopus, *Artocephalus*, *larga* and *virtulina* and so on.

The Caldwells contribute a chapter on behaviour of marine mammals, mainly derived from observations of animals in captivity. They stress the fact that age, sex and individual differences have been largely ignored in cetacean behavioural studies. Another chapter by the same authors on senses and communication is a balanced and cautious review: as they comment "touch is one of the most important senses in dolphins" but "there is an anomaly between anatomy . . . and what the animal demonstrates behaviourally". An interesting discussion containing new material on evolution and cytogenetics by Deborah Kulu illustrates a striking similarity in karyotypes amongst the cetaceans examined but a notable difference in that of *Orcinus*: karyotyping suggests a monophyletic origin for the Pinnipedia. Dailey and Brownell give a checklist of marine mammal parasites and include useful illustrations of representatives of common groups to aid identification. The final and longest chapter, by S. H. Ridgway, is called "Homeostasis in the Aquatic Environment" but in fact is a much wider treatment of general physiology, haematology, pathology, pharmacology, husbandry and veterinary medicine applied to marine mammals. It assembles a formidable amount of information and the results of personal observation and experiment. Some patchiness in the presentation is unavoidable in so vast a coverage and some statements on the anatomy of the ear, reproduction and the lungs will be questioned. This book, and this last chapter in particular, has one highly commendable aspect in its approach in that it informs on how to look after marine mammals in captivity and how to improve their conditions in health and disease.

R. J. HARRISON

How to Plan a Library

The Making of a Library: The Academic Library in Transition. By Robert S. Taylor. Pp. xiii+250. (Becker and Hayes (a Subsidiary of John Wiley): New York and London, March 1972.) £5.25.

THE key to this book rests in a few words which appear only on the dust-jacket as a kind of second sub-title: how to plan dynamic, responsive libraries for the 70s. The working out of a project is described in which Hampshire College, Amherst, designed an extended library, which combined a conventional library, display gallery, AVA centre, bookshop, and a computer centre for both library automation and

reactive teaching. The author, who has been called a meta-librarian, believes that those who plan a modern academic library should first examine its objectives, and what it could do to become a productive participant in the learning process and in the community it serves.

The place of the library in relation to the newer media for transference of information, its inter-relation with educational technology, and its function as a teaching instrument are currently matters of fierce debate, so this is a book of great topical interest. It can be highly recommended because it argues a well-reasoned case from a basis of some practical experience. It thus provides a useful antidote to the dogmatically stated guesswork and to the arrogant staking of territorial claims too frequently found in education journals. Clearly the time has come to recognize the extent of common concern in the library, computer, graphics and communications fields and to solve the problems involved in their proper integration. The ideal is to create a social institution, which will raise the probability of effective use of data, information, knowledge and artistic form in all media in support of education, leisure enjoyment, research and decision making. Taylor avers that "if it cares to make the effort, and if it can make what may be a quantum leap, the library profession, and its social expression in the library, may be the only present institution that can accomplish this".

WILFRED ASHWORTH

Background Cell Biology

Cellular Physiology. By Henry T. Yost. Pp. xiii+925. (Prentice-Hall: Englewood Cliffs, New Jersey, March 1972.) \$14.95.

TEN years ago, even five years ago, it was difficult to find a textbook of cell physiology which could be commended to students for background reading. Now there are a number of such books, but this one by Dr Yost would rate high in most biologists' assessment. It covers a wide field with good sense and criticism, and any student who knew even half of what was in it would have an excellent background for a course in cell biology.

"Cellular physiology," as Dr Yost points out, has no precise meaning. His interpretation is to put a considerable emphasis on cellular biochemistry which occupies about half the book. Another section deals with cell structure. This is adequate, though it might have been more persuasive if a larger number of illustrations had been included. The last quarter of the book is taken up with cell

physiology in the strict sense which is concerned with the behaviour and responses of living cells. This restriction of space means that only some aspects of cell physiology can be covered. The selection that has been made is very reasonable, but it is a pity that some subjects had to be omitted, for example amoeboid movement, the behaviour of nuclear proteins, or radiosensitivity. A reviewer, such as myself, looking at his own speciality might have reservations about its treatment, but he should welcome the clear account even if it was slightly out of date—almost inevitable in a textbook.

This is a big book of nearly a thousand pages, at a price which is not unduly high for its size. I would have hoped, therefore, that it would have a good section of references to original papers. I was disappointed, even though I had taken in the author's statement about the absence of references. Citations do interrupt the flow of reading but there are ways of avoiding this, and, at the level at which this is written, there are good reasons for a student to go to the original papers and try to make judgments about the validity of what is said in the text.

These criticisms, however, are relatively minor, and do not seriously detract from the value of this book. It should prove a most useful contribution to the teaching of cell biology at the level of the graduate student or the advanced undergraduate. J. M. MITCHISON

Trypanosomes

The Trypanosomes of Mammals: a Zoological Monograph. By Cecil A. Hoare. Pp. xvii + 749. (Blackwell Scientific: Oxford and Edinburgh, 1972.) £9.

To the newcomer the literature on trypanosomiasis is formidable, in the first place simply because of its vast size and of the multiplicity of languages in which it is written but more particularly because of its essential miscellaneity. It is still primarily a descriptive literature amassed by investigators widely scattered in the world, often working in isolation, with impure and unstable organismal materials, and heterogeneous hosts. Purification and standardization of organismal materials and the development of comparative experimental work based thereon has not been nearly so much a feature of studies on trypanosomiasis (and indeed of protozoology generally) as it has been of studies in, for example, bacteriology and virology. We are the more fortunate, therefore, that Dr Hoare has brought his immensely long experience of the subject (the earliest paper by him quoted in

the references is 1921) to bear and so has given us a comprehensive and carefully judged distillation of the zoological aspects of the trypanosomiasis.

The book is a culmination of the service for which protozoologists have for long been in debt to Dr Hoare—his continuous logical development of the classification of the trypanosomes of mammals which was begun by Laveran, Duke and Wenyon in the early part of this century. He has now collected together conveniently, and trenchantly discussed, the basic zoological information relating to these organisms. Some 120 pages deal with introductory material—historical, morphological, taxonomic, phylogenetic and ecological—and then the bulk of the remainder, nearly 500 pages, is devoted to a systematic presentation of information species by species. There are three artist-coloured plates of Romanowsky-stained organisms but the main illustration of the work is by line diagrams. These latter compose the bulk of the 108 figures and some ten, fifteen or even more diagrams are given for each species to show the extent of the variation in their form and in their organelles.

Essentially the book is a synthesis of observational and natural history approaches to trypanosomiasis. Diagnostic emphasis is on the recognition of trypanosome species rather than of the infected host. Experimental, biochemical and immunological studies are only briefly mentioned. The most detailed discussions pertain to speciation of trypanosomes, to their evolution and to the epidemiological patterns in which they are transmitted. These discussions are always fascinating though the reader may not always agree with the conclusions reached. The degree of pathogenicity of the organism to its host is frequently advanced as an indication of the antiquity of the relationship between them. Yet this argument is used in both directions; on p. 83 it is argued that the scarcity of *Trypanosoma melophagium* and *T. theodori* in the blood of their mammalian hosts is evidence of their being recent colonizers, still ill-adapted to their vertebrate hosts, while on p. 94 the opposite is contended—that recent association and a low degree of adaptation are indicated by pathogenicity. There are some criticisms: for example, statistically inclined readers will find the presentation of mensural data as ranges, without any indication of variance, unsatisfactory, and comparison of the taxonomy of the organisms with that of their hosts would have been aided by presenting the hosts (in the check list) systematically within orders rather than alphabetically. But these are minor points by comparison with Dr Hoare's signal contribution, that of putting within easy reach of any investigator, and discussing, the litera-

ture relevant to his interest. The book is likely to rival Wenyon's (1926) *Protozoology* in its durability as a reference work for protozoologists.

W. H. R. LUMSDEN

Raman Principles

The Raman Effect. Edited by A. Anderson. Vol. 1. Principles. Pp. ix + 404. (Marcel Dekker: New York, November 1971.) \$28.50.

THIS is the first part of a two-volume work, edited by A. Anderson, concerned with the "principles" of the Raman effect, the latter being interpreted widely to include related phenomena many of which have only been observed since the relatively recent availability of lasers as sources for light scattering experiments. These include the stimulated Raman effect, the inverse Raman effect, the resonance Raman effect, the hyper Raman effect, and also Brillouin scattering and stimulated Brillouin scattering. The book is written at "graduate student" level and, in this volume at least, the emphasis is considerably more on the physics of the subject than on the chemical applications. It is worth emphasizing this, as most books previously published with "Raman effect" in the title have concentrated on the latter aspect.

R. S. Krishnan's first chapter gives an interesting and well balanced historical review of the subject up to the present day. Chantry follows with a clear account of the polarizability theory of the Raman effect, which provides a number of valuable insights. Cowley gives a comprehensive account of the theory of Raman scattering by crystals. This is a subject in which there have been many recent advances; the treatment is also comprehensive but somewhat formal in the mathematical sense. Hathaway gives a good account of present-day Raman instrumentation and techniques, including fairly detailed accounts of the modern spectrometers available on the market. There is here something of a deviation from "principles" and some parts of this chapter will date rather quickly. Lallemand describes the theory and results from the study of the stimulated Raman effect. Krishnan brings the book to a close with a very lucid account of Brillouin scattering, which is an excellent introduction to this subject. This volume does not have an index and suffers for this, but presumably this will be provided at the end of volume 2.

On the whole, however, this volume provides a valuable up-to-date treatment, which is very usefully complementary to previous books on this subject. It is warmly recommended for purchase by physical science libraries.

N. SHEPPARD

CORRESPONDENCE

Science and Politics

SIR,—I recently received, and also saw in *Nature* (237, 469; 1972), a letter from a group at the Open University in Bletchley, England, criticizing me for organizing an Advanced Study Institute on Proteins of the Nervous System under the financial sponsorship of NATO.

The letter states, I believe correctly, that "NATO is primarily a military organization" but "in recent years its activities have ramified in a variety of directions". It then asks whether the deliberations of the meeting are of use for military purposes, if free scientific communication is a permitted consequence, and what the motivations and purposes of the sponsors are.

I was sceptical of sponsorship by an organization with military aims, and was very careful to obtain answers to these same questions, primarily by asking people who had previously organized or participated in such institutes in Neurobiology and Genetics. Only after satisfying myself that no strings were attached did I apply for a grant to NATO. The NATO literature and application forms describe no restraints on who can be invited nor on the content or form of the meeting. The NATO Scientific Affairs Division, which administers the funding of Advanced Study Institutes, strongly urges and almost insists upon independent publication of the proceedings.

Other meetings on the same subject, such as those which have already been held under the sponsorship of the International Society of Neurochemistry and other non-military organizations, are just as likely to lead to military uses of the information discussed. As everyone knows, this is a hazard of basic scientific research in almost any field. Do the signatories to this letter mean to suggest that all meetings relating to the mode of action of the nervous system be forbidden because they might further the development of biological weaponry?

The Open University group also confuses (wittingly or unwittingly) financial support for research and for the purpose of holding meetings. No one can argue intelligently that the purpose of a meeting on proteins of the nervous system, which has fifteen of the world leaders in that field as speakers and which will be openly published, is military. It is absurd to suggest that the organizers, speakers and attendees at the institute are, somehow, being duped by NATO into engaging in a military project.

I do not question the basic motives of the signatories to this letter. I am sure that we agree in our distrust of military organizations and their generally sinister role in our societies. I believe, however, it would be much better to aim at the appropriate targets rather than to shoot wildly at everything in sight, including those projects of NATO which happen to be for a good purpose.

The Open University group sent me a copy of their letter of protest only ten days before our June 1 deadline for receipt of applications—although our meeting had been announced two months before. It was then too late to answer, either publicly or to individuals, the questions they raised. Although we have many more excellent applicants than we can accept from NATO and non-NATO countries, I believe the signatories to the protest letter have been grossly unfair to the organizers of the meeting and to the prospective attendees. Some people who may have wanted to attend may not have applied because they were presented with only one side of the argument.

Finally, and most seriously, such a letter could be bad for the International Society of Neurochemistry (ISN) because it was addressed to ISN members, and it says that the matter will be brought up at the next ISN meeting. The organization could be irreparably harmed by the creation of political factions. At the very least, one of its major advantages, to provide free communication among neurochemists from all countries, could be destroyed.

I implore the writers of this letter to think seriously before they pursue this course any further, risking harm to this great organization for no good purpose. I also urge the members of ISN to resist any attempts by groups such as this, from any country, to introduce irrelevant polarities into the ISN.

Yours faithfully,

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SIR,—As one of the named participants in the NATO Advanced Study Institute on Proteins of the Nervous System, may I make some comments on the letters of the Brain Research Group (BRG) of the Open University (*Nature*, 237, 469; 1972) and Dr Giorgi (*Nature*, 238, 57; 1972).

Dr Giorgi has effectively disposed of the free communication issue as a problem specific to NATO-sponsored conferences. Nevertheless, the point made by the BRG is more generally valid. It is clear, despite the participants from Eastern Europe, that scientists from Cuba or the Democratic Republic of Vietnam would not be welcome at such a meeting. Nor would they be welcome at any meeting held within the United States and most of the American continent. Moreover, the economic and scientific boycott of Cuba, organized by the United States and imposed upon its many allies, is an obvious barrier to free scientific communication. But this can only be opposed by political action to change a political position.

The possible use of scientific meetings to further military research, in particular into CBW, is something which concerns most scientists. Unfortunately, irrespective of the sponsor, the military potential of the work is the same. The decision to be made is not that of participating in certain meetings but whether the research, given its potential, should be carried out. In this, all scientists have an obligation to judge their own work and that of others. Ultimately the problem can only be solved at the political level, and in this context it is worth noting that the movements within the United States against military research have been largely provoked by opponents of the war in Vietnam rather than by scientists objecting only from a general ethical position.

The real problem posed by the BRG is that of whitewashing NATO. This worries me, not because NATO is a military organization, but because it represents the US imperialist system. Participation in NATO conferences can be interpreted as condoning, or at least passively accepting, defoliation, deliberately indiscriminate bombing, anti-personnel weapons of the pineapple bomb type (complete with plastic pellets) and, most recently, deliberate bombing of the system of dams and dykes in the Democratic Republic of Vietnam.

Unlike the Editor of *Nature* (*Nature*, 237, 302 and 238, 57; 1972) I do not feel that scientific objectivity implies silence on these matters because "it is a consequence of a declared military policy of a government". Participating in NATO conferences while opposing the system which it represents certainly leaves me open to the charge of opportunism, but I do not think that boycotting a NATO conference is a practical method of fighting US

imperialism. I prefer to aid the Vietnamese people, as much as I can, in their struggles.

Yours faithfully,

IAN G. MORGAN

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Katchalsky Memorial

SIR,—As scientists we deeply regret Aaron Katchalsky's death and, as friends of the Palestinian people, we fully reprobate the circumstances of that senseless murder. However, the creation of a memorial fellowship to the benefit of the Weizmann Institute is a political gesture which, in the present circumstances, is inadequate and even shocking.

There is no neutral science in a country at war, and this is particularly true for the Israelian state, whose military power is to a very large extent based on advanced technology and high-level scientific manpower. The Weizmann Institute, being the biggest scientific centre of Israel, necessarily plays a key role in that process, even if an important part of the work done there has no direct military purpose and if individuals working at Rehovot are political opponents of the Israelian government. The close links existing between Israelian military and scientific establishments were illustrated, we are sorry to say, by Aaron Katchalsky himself, who, according to *Nature* (237, 301; 1972), was also a leader of the former Hagannah (an anti-Palestinian military organization) and a leading member of the scientific branch of the Israelian army, playing, as you pointed out, "a prominent role in the military affairs" of that country.

These "military affairs" included massacres of the civil population at Deir Yassin and other places, resulted in the expulsion of the Palestinian people from

their own country and, more recently, in military occupation and virtual annexation of territories of three neighbouring lands. Accordingly, we feel that it is rather inopportune, to say the least, to subsidize an official Israelian institution and to provide in that way important moral support to the Israelian government in the name of the international scientific community.

Yours faithfully,

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Announcements

Miscellaneous

ON Monday, July 31, the last seminar held under the auspices of the Institute of Theoretical Astronomy, Cambridge, will be given by Dr S. Aarseth. Its title is "Experimental Numerical Dynamics Is Only Time-dependent Approach".

Reports and Publications

not included in the *Monthly Books Supplement*

Great Britain and Ireland

Advisory Committee on Oil Pollution of the Sea. Annual Report for 1971. Pp. 20. (London: Fauna Preservation Society, c/o Zoological Society of London, 1972.) [56]

Griffin and George. Laboratory Equipment Catalogue 72. Pp. 1073. (Wembley, Middx.: Griffin and George Limited, 1972.) [56]

Department of the Environment. Thirteenth Progress Report of the Standing Technical Committee on Synthetic Detergents. Pp. iv+43. (London: HMSO, 1972.) 35p net. [66]

Fabian Research Series, No. 302: Cuba's Educational Revolution. By Arthur Gillette. Pp. 36. (London: Fabian Society, 1972.) 45p. [66]

Economics and Management in Public Health Engineering. (Proceedings of the Fifth Public Health Engineering Conference held in the Department of Civil Engineering, Loughborough University of Technology, January 1972.) Edited by John Pickford. Pp. 80. (Loughborough: University of Technology, 1972.) £1. [76]

The Grassland Research Institute. Annual Report for 1971. Pp. 142. (Hurley, Maidenhead: The Grassland Research Institute, 1972.) £1.50. [76]

Department of Trade and Industry. Metrication Bibliography: Books and Pamphlets. Pp. 29. (St. Mary Cray, Orpington, Kent: Department of Trade and Industry, Technology Reports Centre, 1972.) 40p net. [76]

The Real Meaning of Academic Freedom. By Sir Robert Birley. Pp. 24. (Edinburgh: Heriot-Watt University Committee of WUS, 1972. Published for World University Service in the United Kingdom.) 30p. [86]

Science Research Council. Space Research Reports, August 1969 to July 31 1970. Pp. 122. (London: HMSO, 1972.) £1.20 net. [86]

Other Countries

US Department of Commerce: National Oceanic and Atmospheric Administration. Atlantic Oceanographic and Meteorological Laboratories. Collected Reprints—1970. Vol. 1: Reprints Nos. 1-41. \$4.50. Vol. 2: Reprints Nos. 42-75. \$4.50. (Washington, DC: Government Printing Office, 1972.) [85]

US Department of the Interior: Geological Survey. Professional Paper 394: Uppermost Precambrian and Lowest Cambrian Rocks in Southeastern Idaho. By Steven S. Oriel and Frank C. Armstrong. Pp. iv+52+plates 1-6. \$2.25. Professional Paper 655-D: Geology and Ground-Water System in the Gila River Phreatophyte Project Area, Graham County, Arizona. By William G. Weist, Jr. Pp. iii+22+plates 1-3. Professional Paper 726-B: Gravity and Magnetic Evidence of Lithology and Structure in the Gulf of Maine Region. By M. F. Kane, M. J. Yellin, K. G. Bell and Isidore Zietz. Pp. iii+22+plates 1 and 2. \$1.25. (Washington, DC: Government Printing Office, 1971 and 1972.) [85]

The Mechanization, Automation, and Increased Effectiveness of the Clinical Laboratory. (A Status Report by the Automation in the Medical Laboratory Sciences-Review Committee of the National Institute of General Medical Sciences, National Institute of Health.) Pp. 78. (Washington, DC: Government Printing Office, 1972.) 70 cents. [95]

Smithsonian Contributions to Paleobiology, No. 11: Homeomorphy in Recent Deep-Sea Brachiopods. By G. Arthur Cooper. Pp. 23 (3 plates). 50 cents. Smithsonian Contributions to Zoology, No. 76: Proceedings of the First International Conference on Meiofauna. Edited by Neil C. Hulings. Pp. viii+205. \$2.25. No. 105: Origins and Affinities of the Troglitic Crayfishes of North America (Decapoda: Astacidae). II. Genus *Orconectes*. By Horton H. Hobbs, Jr., and Thomas C. Barr, Jr. Pp. iii+84. \$1.25. No. 115: Bredin-Archbold-Smithsonian Biological Survey of Dominica: Aculeate Wasps (Hymenoptera: Scolioidea, Vespoidea, Pompiloidea, Sphecoidea). By Howard E. Evans. Pp. 19. 35 cents. No. 118: Studies of Neotropical Caddisflies, XIII: The Genus *Ocatrichia* from Mexico and Central America (Trichoptera: Hydropsychidae). By Oliver S. Flint, Jr. Pp. 28. 40 cents. (Washington, DC: Smithsonian Institution Press, 1971 and 1972. For sale by US Government Printing Office.) [95]

US Department of the Interior: Geological Survey. Bulletin 1354-C: Big Sandy Formation near Wikeup, Mohave County, Arizona. By Richard A. Sheppard and Arthur J. Gude, 3d. Pp. iii+10. 15 cents. Water-Supply Paper 1899-M: Geohydrologic Summary of the Pearl River Basin, Mississippi and Louisiana. By Joseph W. Lang. Pp. iv+44+plates 1 and 2. \$1. Professional Paper 724-B: Normal Fatty Acids in Estuarine and Tidal-Marsh Sediments of Choctawhatchee and Apalachee Bays, Northwest Florida. By Robert E. Miller. Pp. iii+13. 30 cents. (Washington, DC: Government Printing Office, 1972.) [95]

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